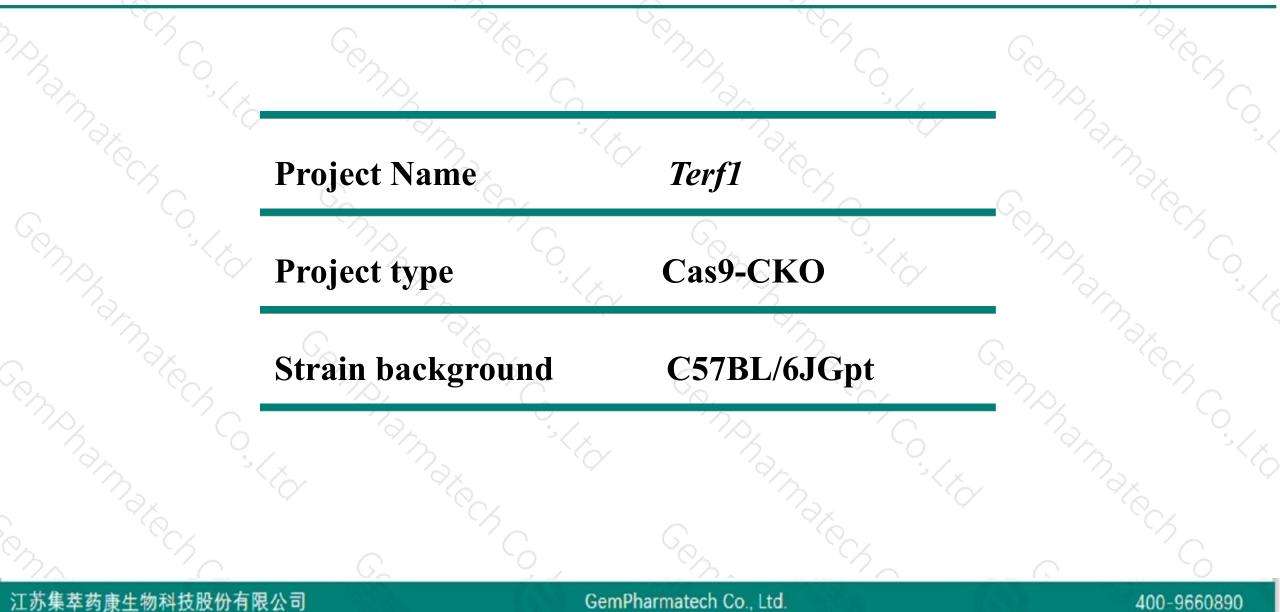


Terf1 Cas9-CKO Strategy

Designer: Xueting Zhang Reviwer:Yanhua Shen Date:2019-10-17

Project Overview

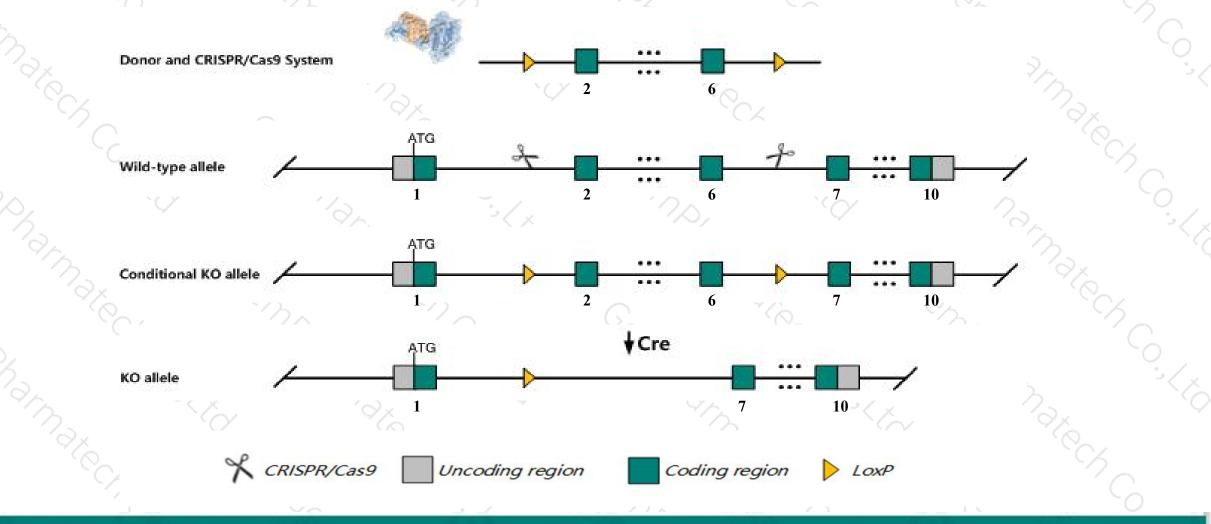




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Terf1* gene. The schematic diagram is as follows:



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The *Terf1* gene has 4 transcripts. According to the structure of *Terf1* gene, exon2-exon6 of *Terf1-203* (ENSMUST00000188371.6) transcript is recommended as the knockout region. The region contains 568bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Terf1* gene. The brief process is as follows:CRISPR/Cas9 system 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 and cell types.

Notice



- According to the existing MGI data, Mice homozygous for disruptions in this gene display embryonic lethality and die sometime before E7.5
- ≻The N-terminal of *Terf1* gene will remain 94aa, it may remain the partial function of *Terf1* gene.
- The *Terf1* gene is located on the Chr1. 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.

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Gene information (NCBI)



\$?

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Terf1 telomeric repeat binding factor 1 [Mus musculus (house mouse)]

Gene ID: 21749, updated on 21-Sep-2019

Summary

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Genomic contex

Official Symbol	Terf1 provided by MGI	
Official Full Name	telomeric repeat binding factor 1 provided by MGI	
Primary source	MGI:MGI:109634	
See related	Ensembl:ENSMUSG0000025925	
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	Pin2; Trf1; Trbf1	
Summary	This gene encodes a protein that binds to repeats in telomeres to form a nucleoprotein complex that protects against the degradation of chromosomal	
	ends. The encoded protein regulates the length of telomeres and is an integral structural component of the functional telomere. This protein is thought	
	to play a role in spindle formation in mitosis. Mutations in this gene are associated with bone marrow failure. Alternative splicing results in multiple	
	transcript variants encoding different isoforms. [provided by RefSeq, Nov 2013]	
Expression	Ubiquitous expression in CNS E11.5 (RPKM 5.8), liver E14 (RPKM 4.7) and 27 other tissues See more	
Orthologs	human all	

Genomic context				
Location: 1 A3; 1 4.88 Exon count: 11	сМ			See Terf1 in Genome Data Viewer
Annotation release	Status	Assembly	Chr	Location
<u>108</u>	current	GRCm38.p6 (GCF_000001635.26)	1	NC_000067.6 (1580554615844052)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	1	NC 000067 5 (15795739 15833510)

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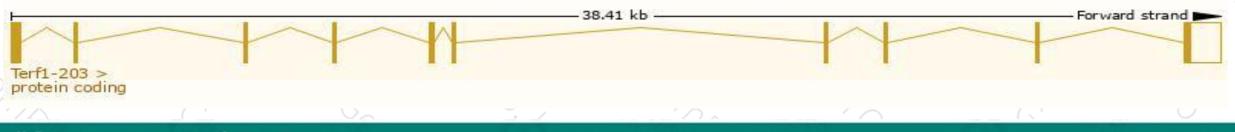
400-9660890

The gene has 4 transcripts, all transcripts are shown below:

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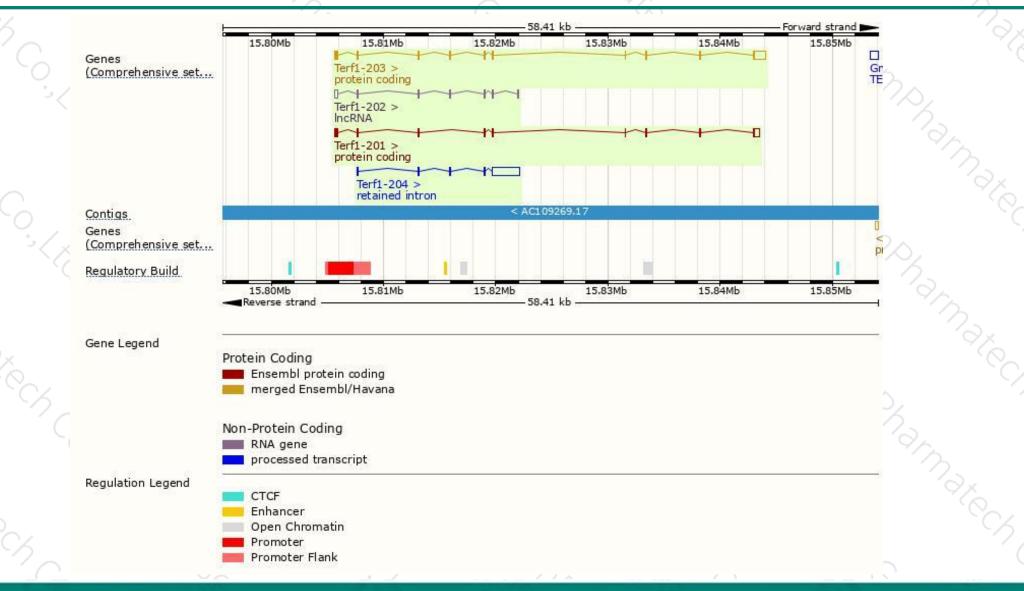
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Terf1-203	ENSMUST00000188371.6	2268	<u>421aa</u>	Protein coding	CCDS14827	P70371	TSL:1 GENCODE basic APPRIS P1
Terf1-201	ENSMUST00000027057.7	1581	<u>392aa</u>	Protein coding	CCDS78547	<u>Q3V252</u>	TSL:1 GENCODE basic
Terf1-204	ENSMUST00000188684.1	2908	No protein	Retained intron	4	84 2 0	TSL:5
Terf1-202	ENSMUST00000186565.6	968	No protein	IncRNA	2	525	TSL:1

The strategy is based on the design of *Terf1-203* transcript, The transcription is shown below



Genomic location distribution



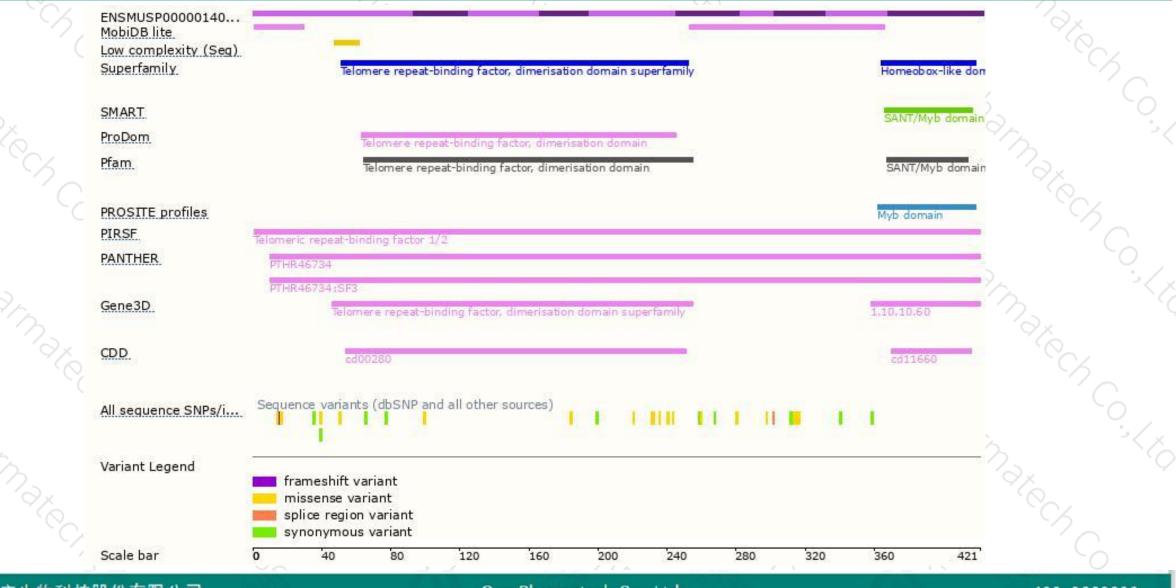


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Protein domain



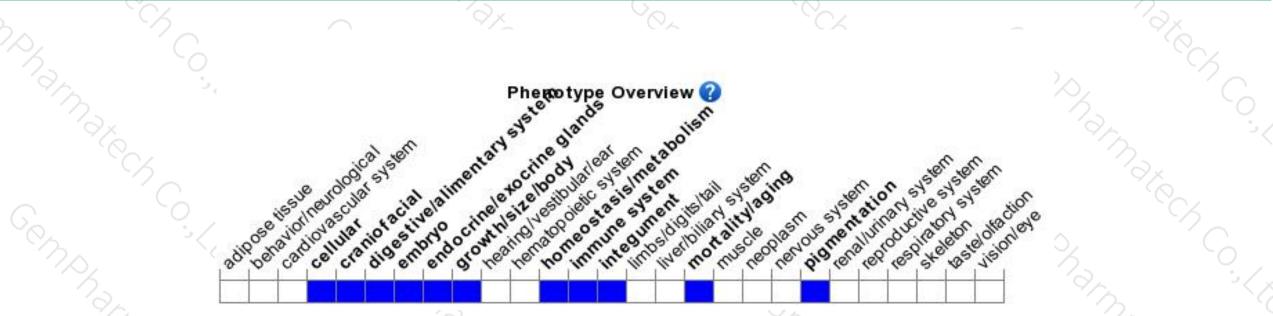


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Mouse phenotype description(MGI)





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 disruptions in this gene display embryonic lethality and die sometime before E7.5



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



