Trim69 Cas9-CKO Strategy

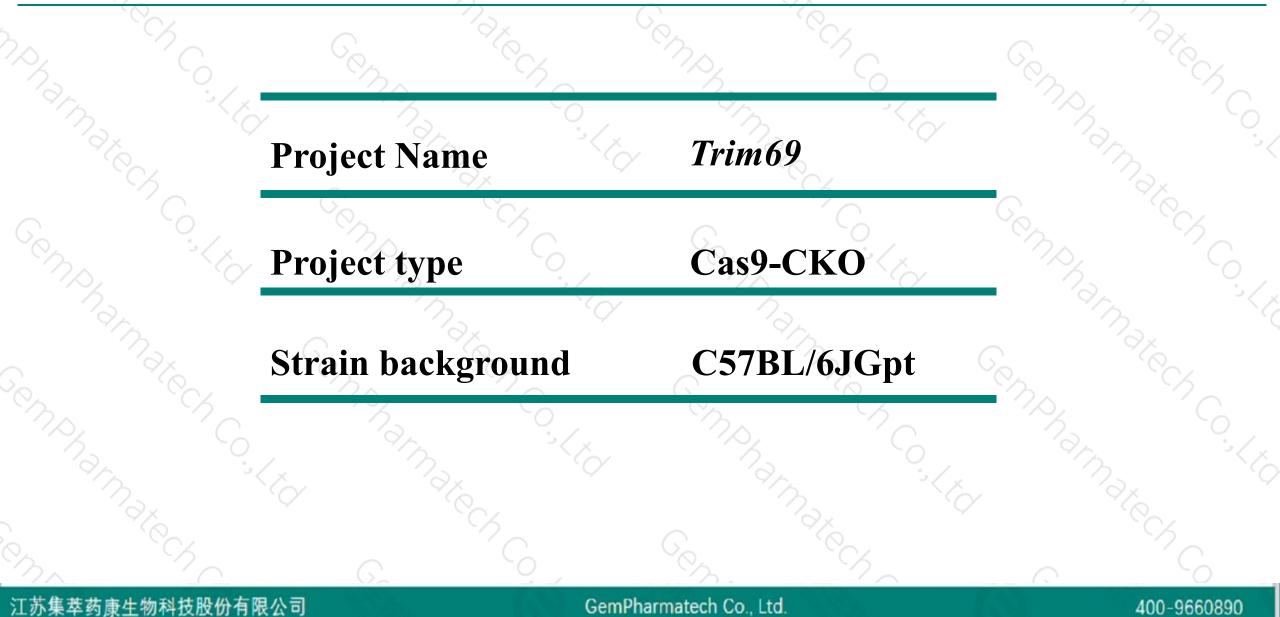
Designer: Reviewer:

Design Date:

Huan Wang Huan Fan 2019-11-19

Project Overview

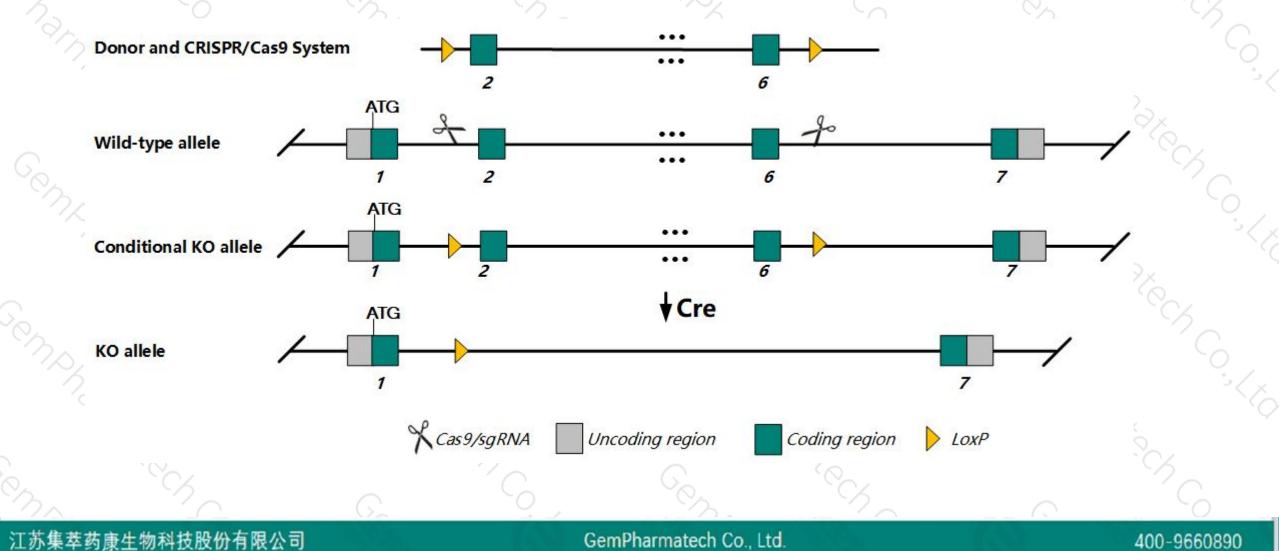




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Trim69 gene. The schematic diagram is as follows:





- The *Trim69* gene has 2 transcript.According to the structure of *Trim69* gene, exon2-6 of *Trim69* 201(ENSMUST00000036089.7) transcript is recommended as the knockout region. The region contains958bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Trim69* 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.

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- The KO region contains functional region of the *Patl2* gene.Knockout the region may affect the function of *Patl2* gene.
 The *Trim69* gene is located on the Chr2. 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.

Notice

Gene information (NCBI)



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Trim69 tripartite motif-containing 69 [Mus musculus (house mouse)]

Gene ID: 70928, updated on 12-Aug-2019

Summary

Official SymbolTrim69 provided by MGIOfficial Full Nametripartite motif-containing 69 provided by MGIPrimary sourceMGI:MGI:1918178See relatedEnsembl:ENSMUSG0000033368Gene typeprotein codingrefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae;
Murinae; Mus; MusAlso knowa seTif, Rnf36; Trimless; 4921519C19RikExpressionRestricted expression toward testis adult (RPKM 46.9) See more
human all

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400-9660890

Transcript information (Ensembl)



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

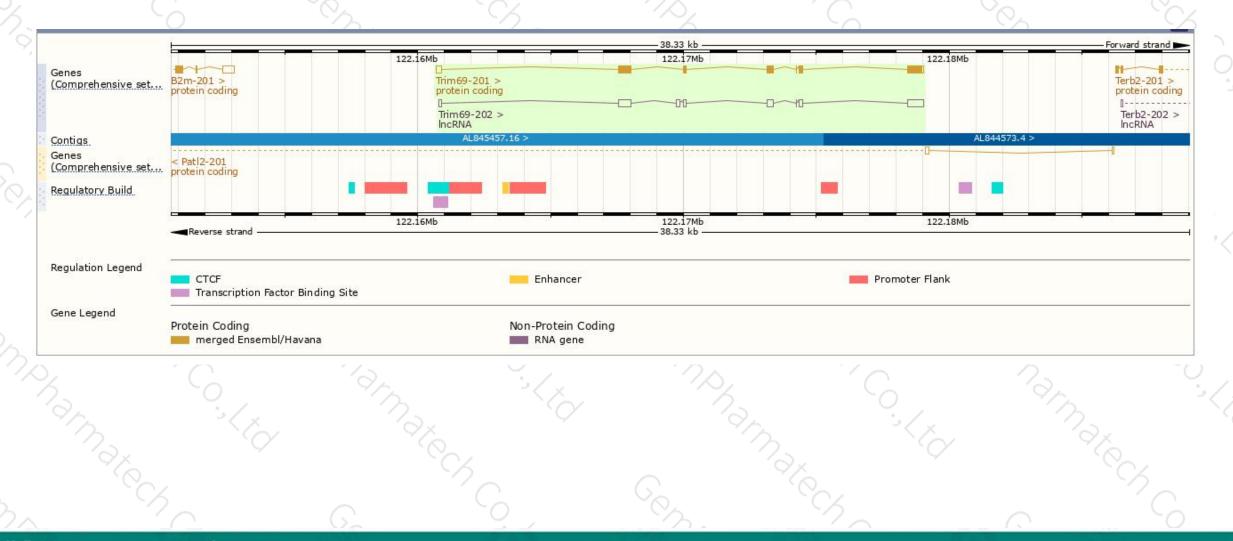
Name 🖕	Transcript ID	bp 🖕	Protein 🖕	Biotype 🍦	CCDS 🖕	UniProt	Flags 👙			
Trim69-201	ENSMUST0000036089.7	1758	<u>500aa</u>	Protein coding	<u>CCDS16655</u> 료	<u>Q80X56</u> 교	TSL:1	GENCODE basic	APPRIS P1	
Trim69-202	ENSMUST00000143088.1	1783	No protein	IncRNA	22	-		TSL:5		

The strategy is based on the design of *Trim69*-201 transcript, The transcription is shown below



Genomic location distribution





Protein domain



:<⊠%5										
ENSMUSP00000047 Low complexity (Seg) Coiled-coils (Ncoils) Superfamily	SSF578	50				Concar	navalin A-like lectin/glucanase	e domain superfamily		
SMART.	Zin	c finger, RING-type				SPRY-a	ssociated SPRY doma	ain		
Prints. Pfam	PF1	5227					And and an and an	ain	=	
PROSITE profiles	Zin	c finger, RING-type				B30.2/SPRY do	main			
PROSITE patterns PANTHER	PTHR24103:SF286	Zinc finger, RING-ty	rpe, conserved site						_	
Gene3D	Zinc finger, RING/FYVE/PHD-type 3.30.40.200				2.60.120.920					
All sequence SNPs/i	Sequence variants (dbSNP and all other s	sources)			11 i i i	T T	1 Y	î.	
Variant Legend	inframe deletion synonymous variant			🦰 missense vari	ant	5				
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If you have any questions, you are welcome to inquire. Tel: 400-9660890



