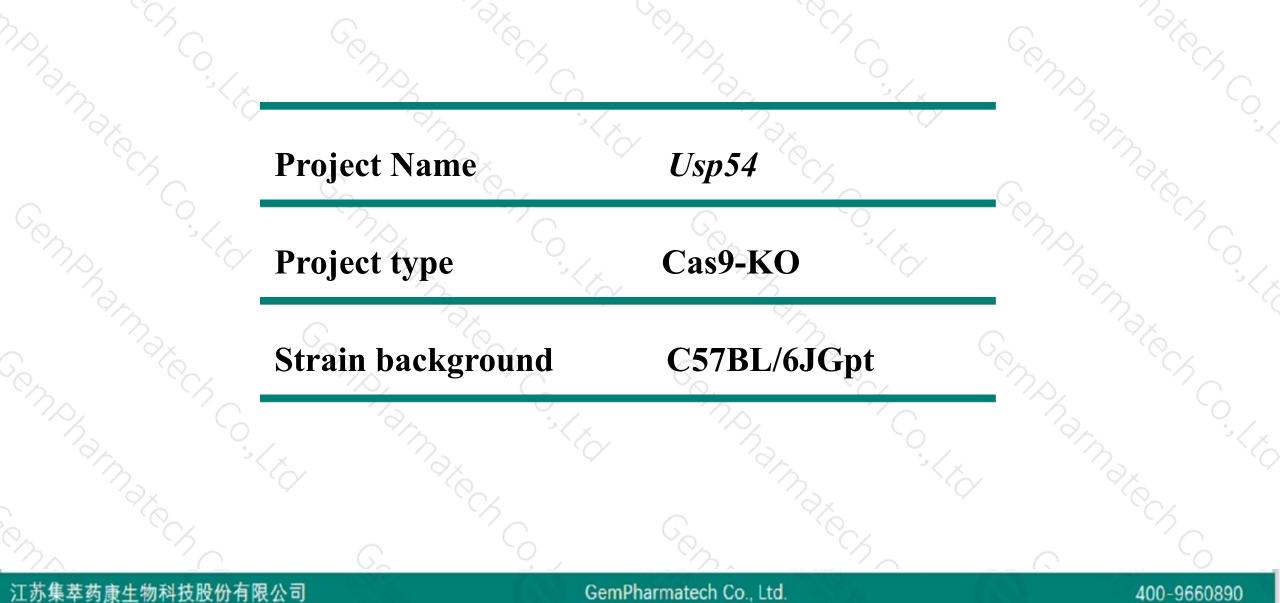


Usp54 Cas9-KO Strategy

Designer: Reviewer: Design Date: Baocheng Zhuang Yang Zeng 2018-6-8

Project Overview

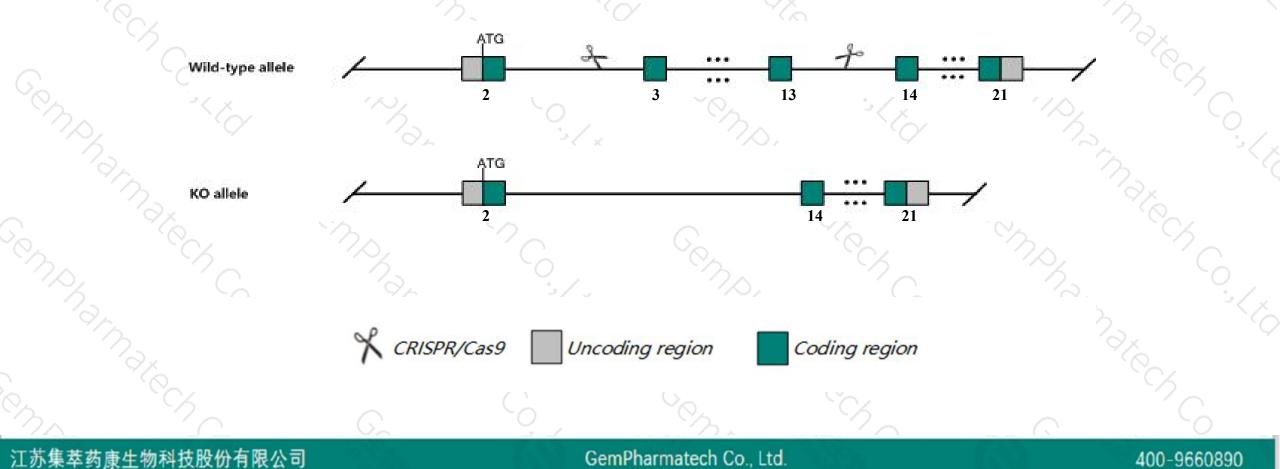




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Usp54* gene. The schematic diagram is as follows:





- The Usp54 gene has 5 transcripts. According to the structure of Usp54 gene, exon3-exon13 of Usp54-202 (ENSMUST00000035340.13) transcript is recommended as the knockout region. The region contains 1739bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify Usp54 gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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 KO region is close to 3'UTR region of the 1810062018Rik gene. Knockout the region may affect the expression of 1810062018Rik gene.
- The Usp54 gene is located on the Chr14. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



2

Usp54 ubiquitin specific peptidase 54 [Mus musculus (house mouse)]

Gene ID: 78787, updated on 12-Aug-2019

Summary

Official Symbol Usp54 provided by MGI Official Full Name ubiquitin specific peptidase 54 provided by MGI Primary source MGI:MGI:1926037 See related Ensembl:ENSMUSG0000034235 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 AI115571; 4930429G18Rik; C030002J06Rik Also known as Ubiquitous expression in cerebellum adult (RPKM 6.3), cortex adult (RPKM 4.8) and 27 other tissues See more Expression Orthologs human all

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Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below: Name 🔺 Transcript ID Protein Translation ID Biotype CCDS UniProt Flags bp Usp54-201 ENSMUST0000022356.11 1588aa ENSMUSP00000022356.4 Protein coding **GENCODE** basic **APPRIS P1** 6580 CCDS26847 Q8BL06译 TSL:5 Usp54-202 ENSMUST0000035340.13 6495 1588aa ENSMUSP0000036214.7 Protein coding CCDS26847 🖗 Q8BL06 TSL:5 GENCODE basic APPRIS P1 Usp54-203 ENSMUST00000123287.1 666 222aa ENSMUSP00000117503.1 Protein coding F6ZXV1函 CDS 5' and 3' incomplete TSL:3 Retained intron Usp54-204 ENSMUST00000127342.1 1980 No protein TSL:1 Retained intron 3423 No protein Usp54-205 TSL:1 ENSMUST00000143267.

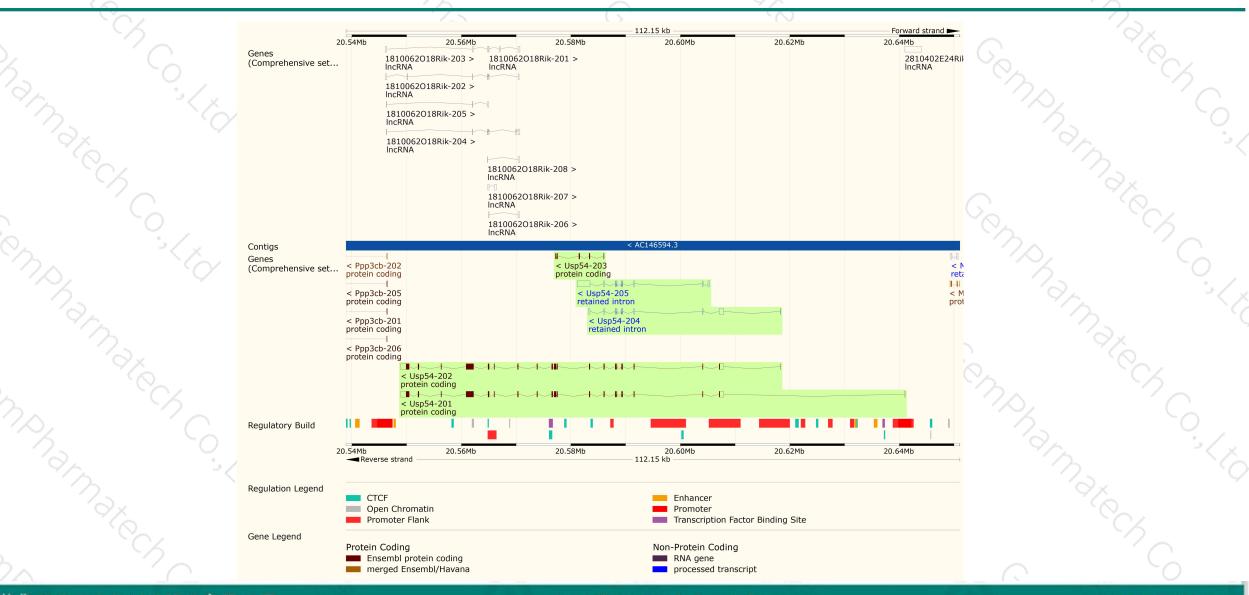
69.44

The strategy is based on the design of Usp54-202 transcript, The transcription is shown below

< Usp54-202 protein coding

Reverse strand

Genomic location distribution



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Protein domain



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<.	ENSMUSP00000036 MobiDB lite Low complexity (Seg) Superfamily	Papain-like cysteine p	peptidase superfamily	y					_	
	Pfam	Peptidase C19, ubiqu								
	PROSITE profiles	Ubiquitin specific prot	cease domain							
	PANTHER	Inactive ubiquitin carbox	yl-terminal hydrolase	2 5 4						
G	Gene3D	PTHR22975 3.90.70.10								
	CDD	cd02257								
	All sequence SNPs/i	Sequence variants (dl	SNP and all other	sources)						
	All sequence SNFS/1	111-1							1 1	- 4
	Variant Legend	splice donor vari missense varian	iant			inframe inser	rtion			<
		splice donor vari missense varian	iant	400	600	inframe inser	rtion	1200		1588
	Variant Legend	splice donor vari missense varian	iant t	I <u>I</u> II		inframe inser	rtion variant			1588

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



