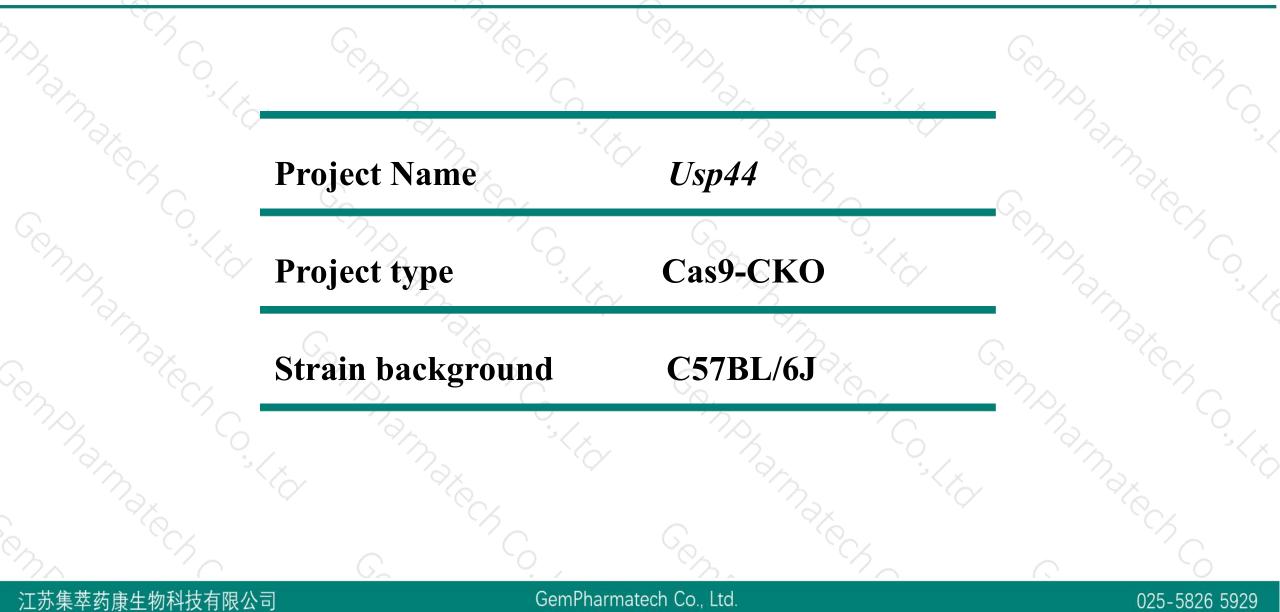


Usp44 Cas9-CKO Strategy

Designer: Xiaojing Li Design Date: 2019-9-19 Reviewer: JiaYu

Project Overview

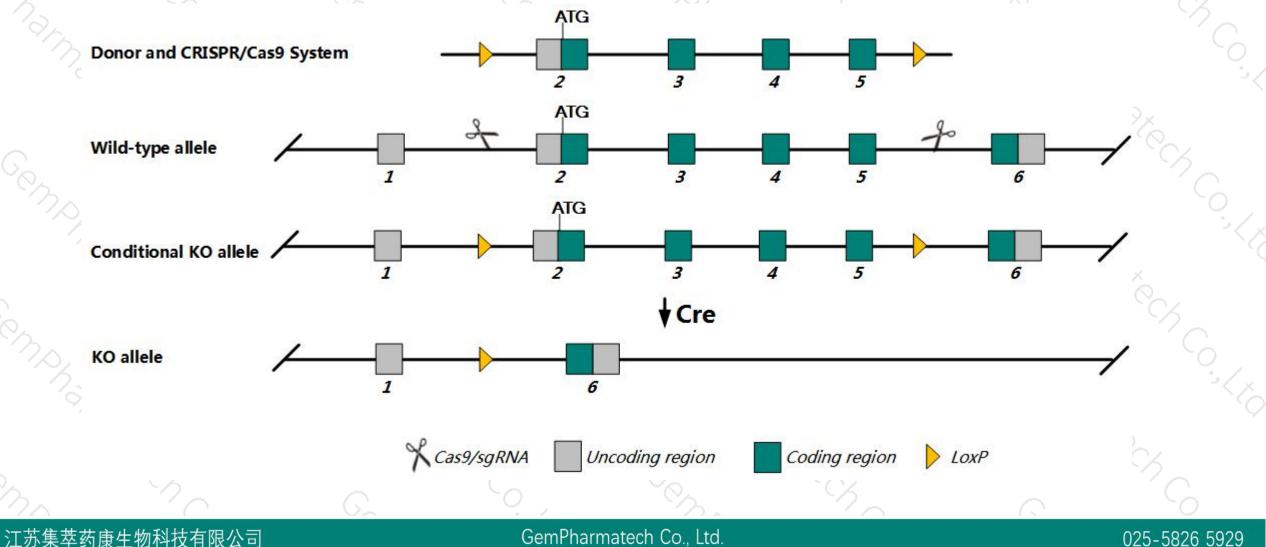




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Usp44* gene. The schematic diagram is as follows:





The Usp44 gene has 2 transcripts. According to the structure of Usp44 gene, exon2-exon5 of Usp44-202 (ENSMUST00000216224.1) transcript is recommended as the knockout region. The region contains ATG initiation codon. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify Usp44 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/6J 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/6J 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 Usp44 gene is located on the Chr10. 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.
- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit chromosomal instability, aneuploidy and increased tumor incidence.
- 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)



025-5826 5929

Usp44 ubiquitin specific peptidase 44 [Mus musculus (house mouse)]

Gene ID: 327799, updated on 21-Aug-2019

Summary

Official Symbol	Usp44 provided by MGI
Official Full Name	ubiquitin specific peptidase 44 provided by MGI
Primary source	MGI:MGI:3045318
See related	Ensembl:ENSMUSG0000020020
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	E430004F17Rik
Expression	Biased expression in testis adult (RPKM 24.5), CNS E11.5 (RPKM 1.5) and 1 other tissue See more
Orthologs	human all

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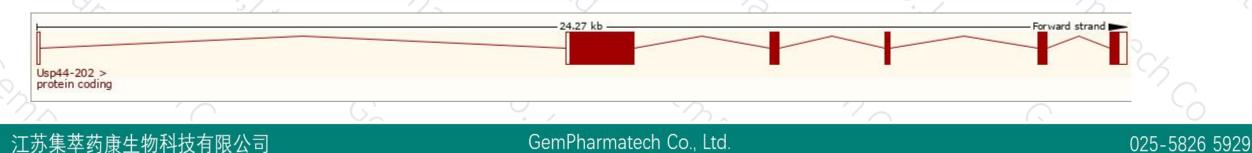
Transcript information (Ensembl)



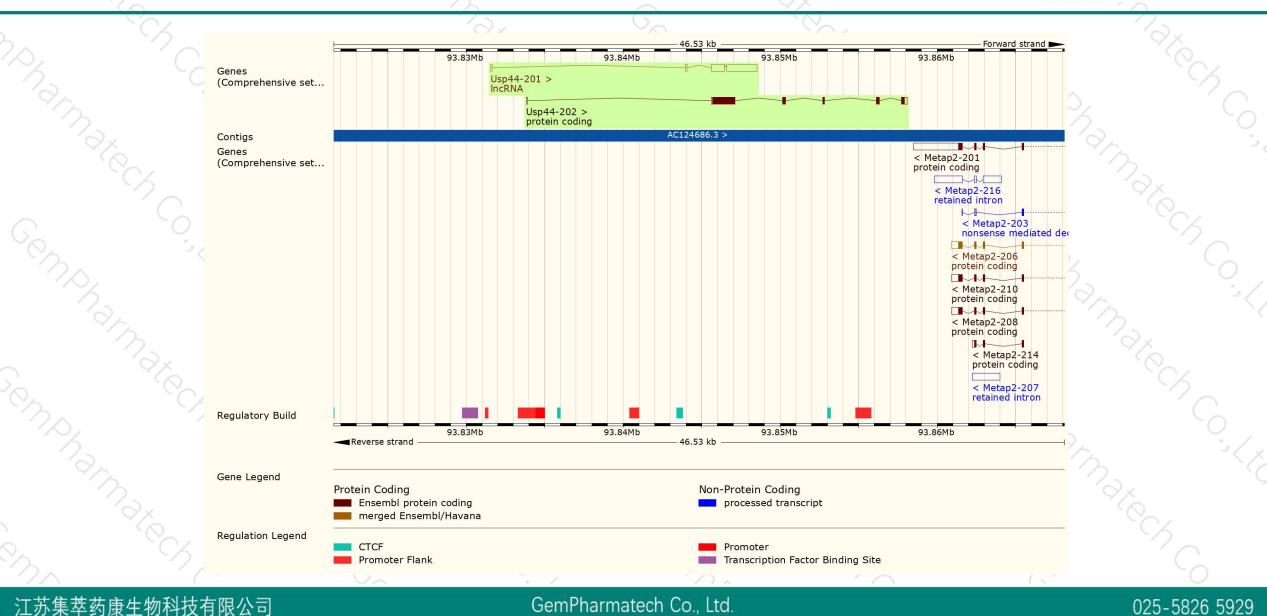
The gene has 2 transcripts, all transcripts are shown below:

Name 🔺	Transcript ID 🖕	bp 🖕	Protein 🖕	Biotype 🍦	CCDS 🖕	UniProt 🖕	Flags 🖕
Usp44-201	ENSMUST0000095333.5	2946	No protein	IncRNA	-	878	TSL:1
Usp44-202	ENSMUST00000216224.1	2449	<u>711aa</u>	Protein coding	-	<u>Q8C2S0</u> ₽	TSL:5 GENCODE basic APPRIS P1

The strategy is based on the design of Usp44-202 transcript, The transcription is shown below



Genomic location distribution



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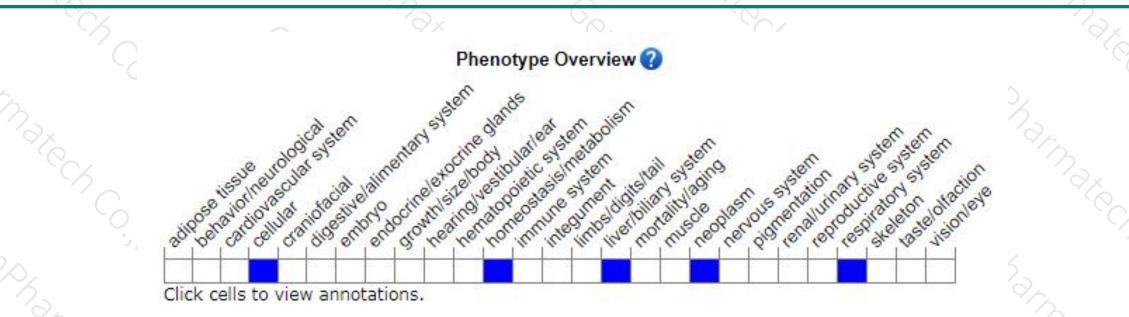
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Protein domain



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(\mathcal{D})	Variant Legend	inframe deletion synonymous varia	nt) 57 g				
	All sequence SNPs/i	Sequence variants (dbS	NP and all other so	ources)	1 110		11	3		1	
	Gene3D	Zinc finger, RING/FYVE/PHI			3.90.70.10	ł					
	PANTHER	PTHR21646 PTHR21646:SF15									
~/,	PROSITE patterns				Ubiquitin specific pr	rotease, conserv	ved site		Ubio	quitin specific protease,	0./
Co,	PROSITE profiles	Zinc finger, UBP-type	2		Ubiquitin specific pr	otease domain					
	Pfam	Zinc finger, UBP-typ	e		Peptidase C19, ubiq	quitin carboxyl-te	erminal hydrolase				
	SMART	Zinc finger, UBP-type	e								
6	Coiled-coils (Ncoils) Superfamily	SSF57850		_	Papain-like cysteine	peptidase super	family				S
\sim	ENSMUSP00000149 MobiDB lite Low complexity (Seg)		-			_		_			
										100	

Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

Mice homozygous for a knock-out allele exhibit chromosomal instability, aneuploidy and increased tumor incidence.



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



