

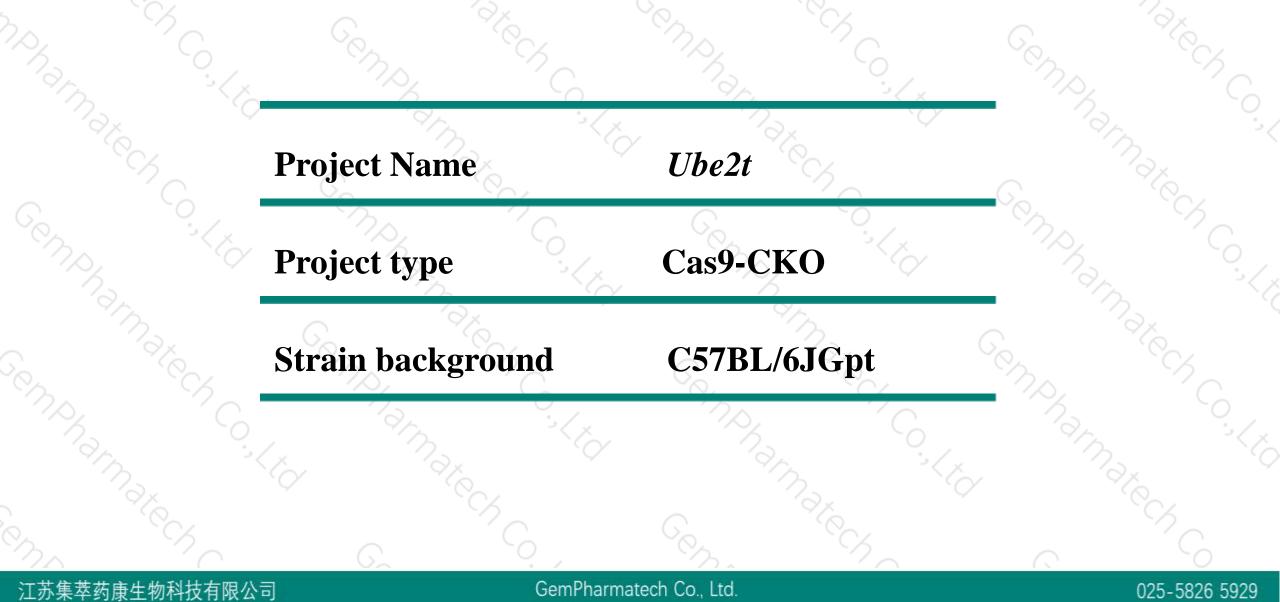
Ube2t Cas9-CKO Strategy andramater Contra

empharmatect

Cempharmatech Cemphamatech, Designer: Yanhua Shen Design Date: 2019-08-05

Project Overview

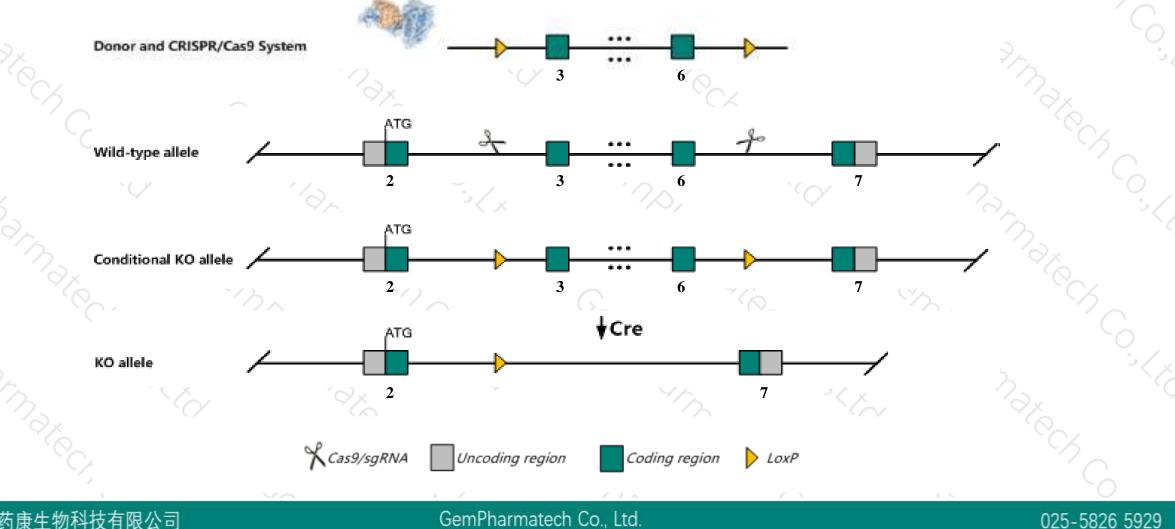




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Ube2t* gene. The schematic diagram is as follows:



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- The Ube2t gene has 5 transcripts. According to the structure of Ube2t gene, exon3-exon6 of Ube2t-201
 (ENSMUST00000027687.7) transcript is recommended as the knockout region. The region contains 359bp coding sequence.
 Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify Ube2t 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.

Notice



- The Ube2t 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.



Gene information (NCBI)



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Ube2t ubiquitin-conjugating enzyme E2T [Mus musculus (house mouse)]

Gene ID: 67196, updated on 31-Jan-2019

Summary

Official Symbol	Ube2t provided by MGI						
Official Symbol	obezt provided by Mor						
Official Full Name	ubiquitin-conjugating enzyme E2T provided by <u>MGI</u>						
Primary source	<u>MGI:MGI:1914446</u>						
See related	Ensembl:ENSMUSG0000026429						
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	2700084L22Rik, C80607						
Expression	Biased expression in testis adult (RPKM 20.3), liver E14 (RPKM 12.2) and 14 other tissuesSee more						
Orthologs	human all						

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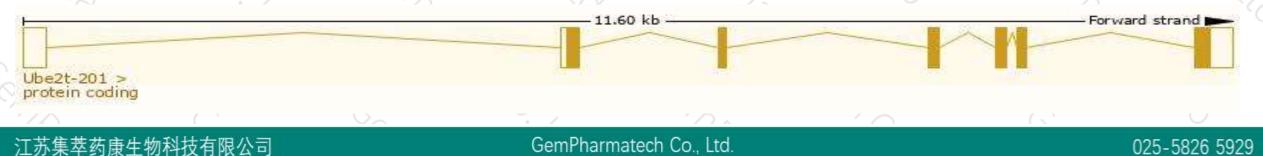
Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

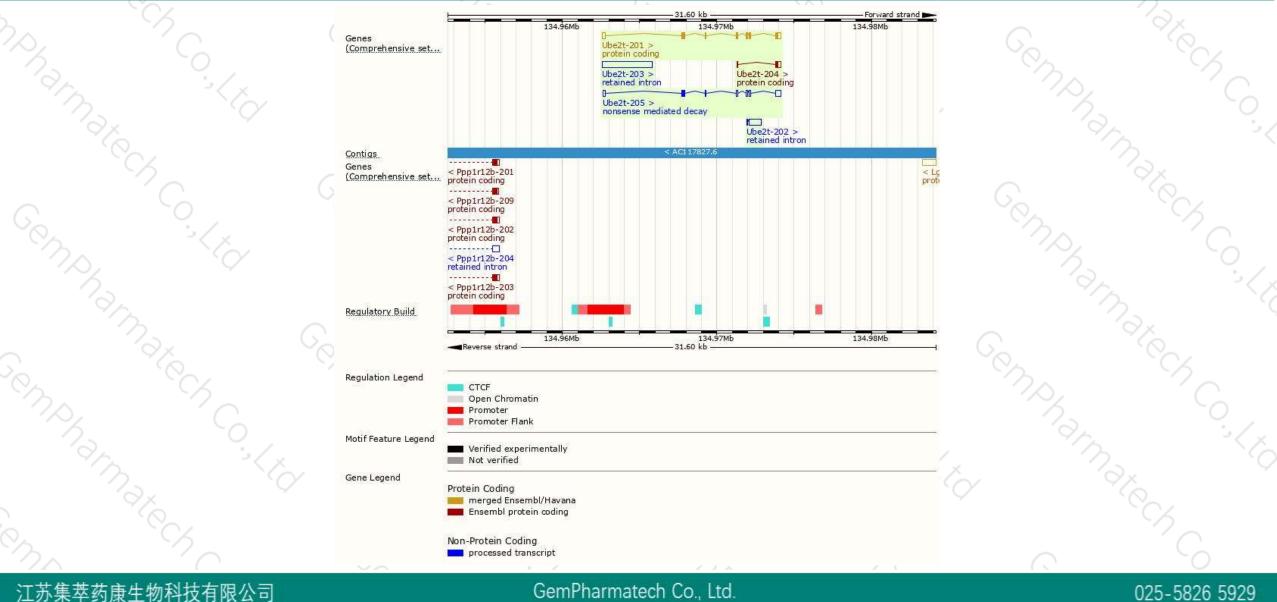
					1 2		
Name	Transcript ID	bp	Protein	Biotype	CCDS UniProt		Flags
Ube2t-20	ENSMUST0000027687.7	1116	<u>204aa</u>			<u>Q9CQ37</u>	TSL:1 GENCODE basic APPRIS P1
Ube2t-204	ENSMUST00000188177.1	437	<u>74aa</u>			A0A087WPV1	CDS 5' incomplete TSL:5
Ube2t-20	5 ENSMUST00000223886.1	1046	<u>43aa</u>	Nonsense mediated decay	-	A0A286YCR4	
Ube2t-20	B ENSMUST00000185369.1	3244	No protein	Retained intron	-	-	TSL:NA
Ube2t-202	ENSMUST00000139617.1	796	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of Ube2t-201 transcript, The transcription is shown below



Genomic location distribution





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Protein domain



	с Х С	Geo	A CA	CON X),),	°%		G	Nate C	2
	ENSMUSP00000027 MobiDB lite Conserved Domains hmmpanther	PTHR44511					-			26.
	Superfamily domains SMART domains	SM00212	ng enzyme/RWD-like							
	Pfam_domain PROSITE profiles PROSITE patterns	Ubiquitin-conjuga Ubiquitin-conjuga		Ubiquitin-cor	ijugating enzy	me, active site				Co.<
	Gene3D All sequence SNPs/i Variant Legend		g enzyme/RWD-like (dbSNP and all oth	ner sources)	1		a.		<u>i u</u>	
5		stop lost missense var synonymous	variant	122	101212		Constant.	1		-367
	Scale bar	o 20	40 60	80	100	120	140	160	180 204	

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



