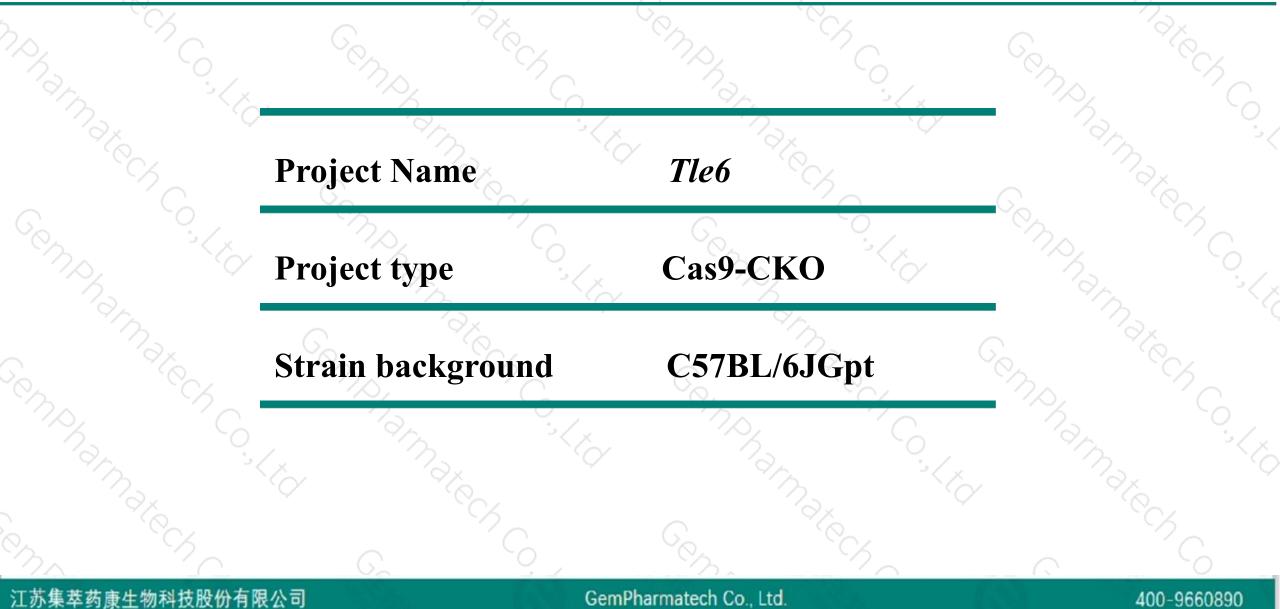


# **Tle6 Cas9-CKO Strategy**

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2020-02-19

# **Project Overview**



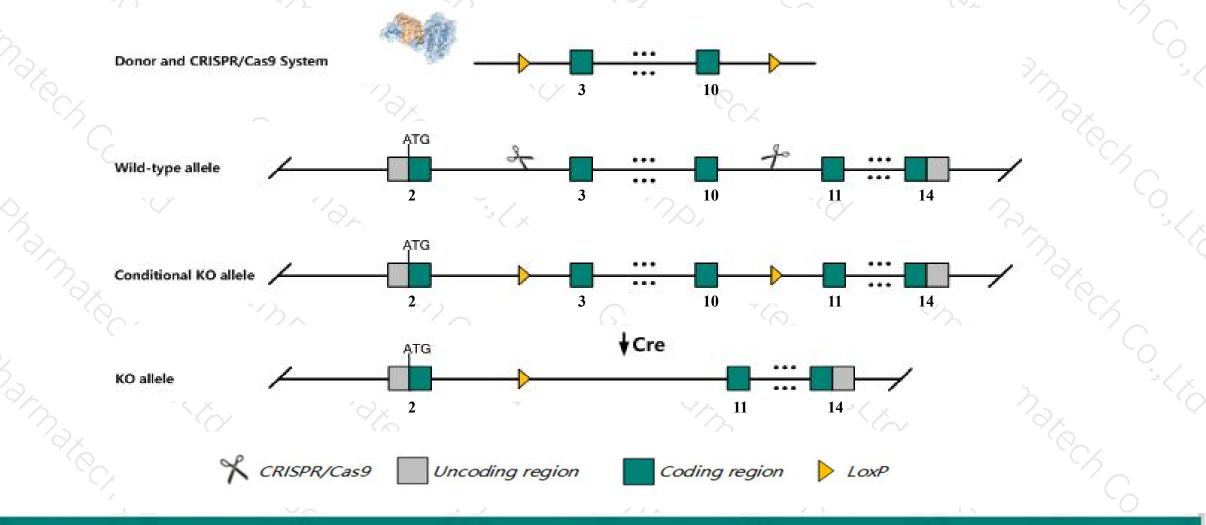


# **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the *Tle6* gene. The schematic diagram is as follows:



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The *Tle6* gene has 14 transcripts. According to the structure of *Tle6* gene, exon3-exon10 of *Tle6-201* (ENSMUST00000072020.8) transcript is recommended as the knockout region. The region contains 1223bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Tle6* 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



- The floxed region is near to the N-terminal of BC025920 gene, this strategy may influence the regulatory function of the N-terminal of BC025920 gene.
- ≻Transcript *Tle6*-205&211 may not be affected.
- > The *Tle6* 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.
- 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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 Tle6
 transducin-like enhancer of split 6 [ Mus musculus (house mouse) ]

 Gene ID: 114606, updated on 14-Aug-2019

 Image: Summary

 Official Symbol
 Tle6 provided by MGI

 Official Full Name
 transducin-like enhancer of split 6 provided by MGI

 Official Full Name
 transducin-like enhancer of split 6 provided by MGI

 Primary source
 MGI:MGI:2149593

 See related
 Ensembl:ENSMUSG00000034758

 Gene type
 protein coding

 RefSeq status
 VALIDATED

 Organism
 Mus musculus

 Lineage
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Murinae; Mus; Mus

 Also known as
 Grg6; 1810057E06Rik

 Expression
 Broad expression in ovary adult (RPKM 24.8), adrenal adult (RPKM 10.1) and 17 other tissues <u>See more</u>

 Orthologs
 human all

#### Genomic context

Location: 10; 10 C1

See Tle6 in Genome Data Viewer

Exon count: 16

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	10	NC_000076.6 (8159090481601185, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	10	NC_000076.5 (8105365081063645, complement)

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# **Transcript information (Ensembl)**

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G	集萃药康 GemPharmatech

Name 🖕	Transcript ID	bp 🖕	Protein 🖕	Biotype 🖕	CCDS	UniProt	Flags		\$
Tle6-201	ENSMUST0000072020.8	2015	<u>581aa</u>	Protein coding	<u>CCDS24064</u> &	<u>Q9WVB3</u> &	TSL:1 GENC	ODE basic AF	PRIS P3
Tle6-210	ENSMUST00000142948.7	1961	<u>571aa</u>	Protein coding	<u>CCDS83737</u> &	<u>D3Z5E1</u> &	TSL:1 GENCO	DE basic API	PRIS ALT2
Tle6-213	ENSMUST00000151858.7	627	<u>101aa</u>	Protein coding		<u>D6RI70</u> &	TSL:3	GENCODE bas	sic
Tle6-203	ENSMUST00000127546.1	544	<u>137aa</u>	Protein coding	-	<u>D3YV26</u> &	CDS 3' in	ncomplete TS	L:3
Tle6-209	ENSMUST00000137631.7	1972	No protein	Processed transcript	<u>.</u>	<u>-</u>		TSL:1	
Tle6-204	ENSMUST00000128278.7	1855	No protein	Processed transcript	-	5		TSL:1	
le6-212	ENSMUST00000149721.1	623	No protein	Processed transcript	-	-		TSL:3	
Tle6-206	ENSMUST00000131530.7	620	No protein	Processed transcript	-			TSL:2	
Tle6-205	ENSMUST00000129282.7	469	No protein	Processed transcript	-	Ļ		TSL:2	
le6-208	ENSMUST00000135008.1	375	No protein	Processed transcript	-	<u>-</u>		TSL:2	
le6-214	ENSMUST00000153379.7	1246	No protein	Retained intron		7		TSL:1	
Tle6-202	ENSMUST00000124724.7	767	No protein	Retained intron		-		TSL:3	
le6-207	ENSMUST00000134457.1	620	No protein	Retained intron	( <u>1</u> )	÷		TSL:2	
Fle6-211	ENSMUST00000146239.1	464	No protein	Retained intron	-	Ļ		TSL:3	

The gene has 14 transcripts all transcripts are shown below.

The strategy is based on the design of *Tle6-201* transcript, The transcription is shown below

< Tle6-20 protein codina

everse strand

10.00 kb

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### **Genomic location distribution**

81.60Mb

Open Chromatin

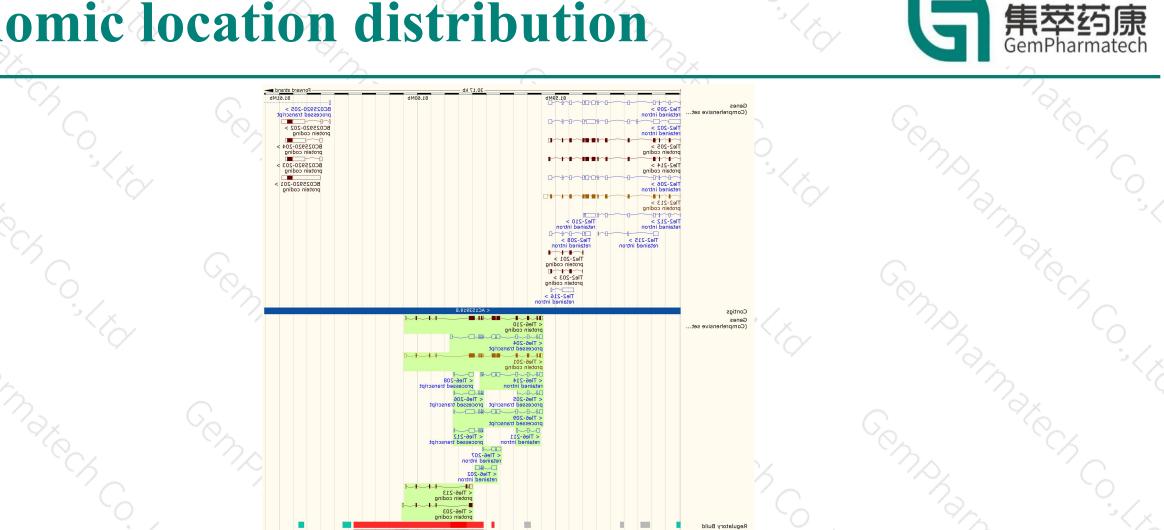
Promoter Flank

Non-Protein Coding

processed transcript

30.17 kb

81.61Mb



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81.59Mb

Reverse strand

CTCF

Promoter

Protein Coding

merged Ensembl/Havana Ensembl protein coding Regulation Legend

Gene Legend

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### **Protein domain**



noparnax	So.	Cons.			%	х Кор	G		19
3	ENSMUSP00000071 MobiDB lite	-	_					_	
×6.	Superfamily					taining domain superfamily			
5	SMART				WD40 repeat	-		-	5
	PROSITE profiles					WD40 rep			
G	PANTHER	Groucho/transducin-like enhar	icer			WD40-re	peat-containing domain	n	18
Sho.	Gene3D	PTHR10814:SF2		WD40/	YVTN repeat-like-c	containing domain superfam	nily		
Comphan,	All sequence SNPs/i	Sequence variants (dbSNP	and all other sources)	$\{i,j\} = 1$	1.1.10	1	1 = 1.11	$\{1, 2\}$	
	Variant Legend	inframe deletion synonymous variant			missense	e variant			- X
~~ [	Scale bar	<b>o</b> 60	120 180	240	300	360 420	480	58	1
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If you have any questions, you are welcome to inquire. Tel: 400-9660890



