

# Sgta Cas9-CKO Strategy

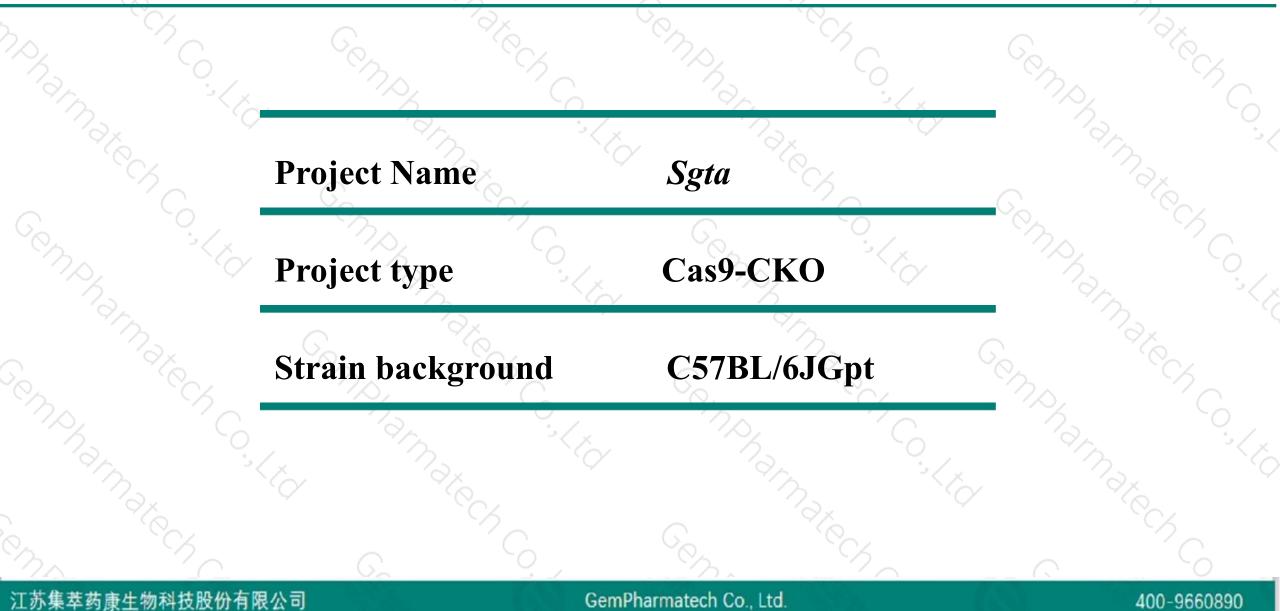
Designer: Reviewer:

**Design Date:** 

Huan Wang Huan Fan 2020-4-22

# **Project Overview**

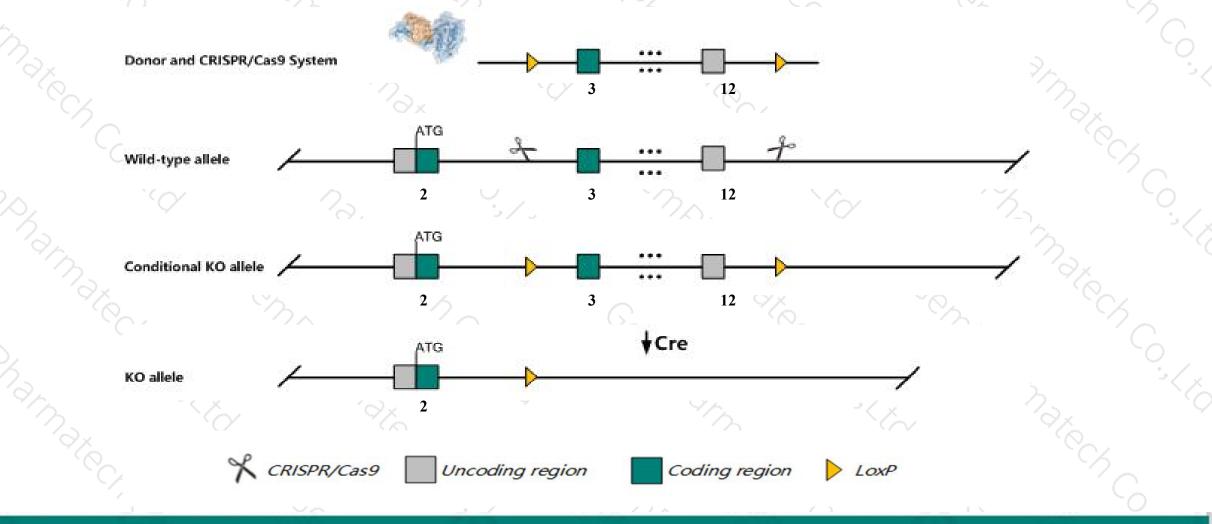




# **Conditional Knockout strategy**



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



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The Sgta gene has 7 transcripts. According to the structure of Sgta gene, exon3-exon12 of Sgta-201 (ENSMUST0000005067.5) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Sgta* 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 and cell types.



- According to the existing MGI data,mice homozygous for a knock-out allele exhibit increased neonatal death, subfertility, decreased body size, increased brain, intestine and heart weight, decreased serum insulin-like growth factor i level, large penis, and increased testis descent.
- The Sgta 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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### Sgta small glutamine-rich tetratricopeptide repeat (TPR)-containing, alpha [Mus musculus (house mouse)]

Gene ID: 52551, updated on 13-Mar-2020

### Summary

Official Symbol	Sgta provided by MGI									
Official Full Name										
Primary source	IGI:MGI:1098703									
See related	Ensembl:ENSMUSG0000004937									
Gene type	protein coding									
<b>RefSeq status</b>	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	5330427H01Rik, Al194281, D10Ertd190e, Sgt, Stg									
Expression	Ubiquitous expression in adrenal adult (RPKM 191.2), mammary gland adult (RPKM 106.5) and 28 other tissues See more									
Orthologs	human all									

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# **Transcript information (Ensembl)**



### The gene has 7 transcripts, all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sgta-201	ENSMUST0000005067.5	1969	<u>315aa</u>	Protein coding	CCDS35990	<u>Q8BJU0</u>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Sgta-202	ENSMUST00000218208.1	1964	<u>314aa</u>	Protein coding	-	Q3TN35 Q8BJU0	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT 1
Sgta-205	ENSMUST00000219401.1	869	<u>211aa</u>	Protein coding	25	A0A1W2P6P1	CDS 3' incomplete TSL:2
Sgta-207	ENSMUST00000220317.1	775	<u>166aa</u>	Protein coding	20	A0A1W2P7I5	CDS 3' incomplete TSL:3
Sgta-203	ENSMUST00000219126.1	698	No protein	Retained intron		-	TSL:5
Sgta-204	ENSMUST00000219297.1	693	No protein	Retained intron	-	<del>.</del>	TSL:3
Sgta-206	ENSMUST00000220202.1	642	No protein	Retained intron	-	20	TSL:2
	- mer		-		V 2.		

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

< Sgta-201 protein coding

Reverse strand

- 16.10 kb -

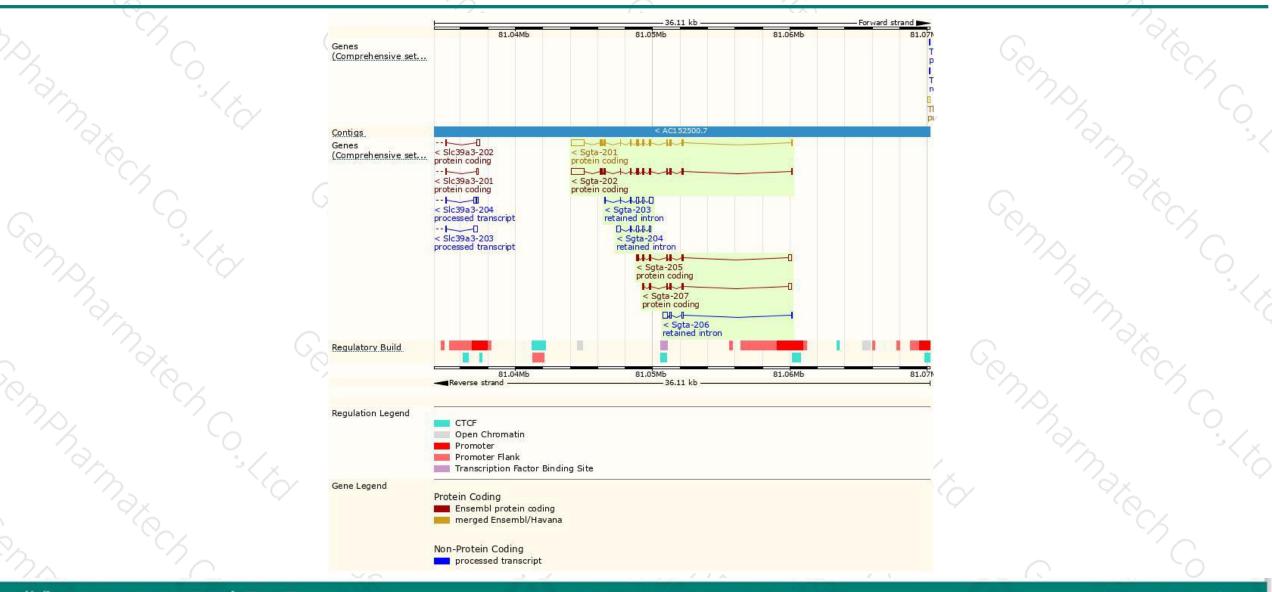
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## **Genomic location distribution**



400-9660890



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# **Protein domain**

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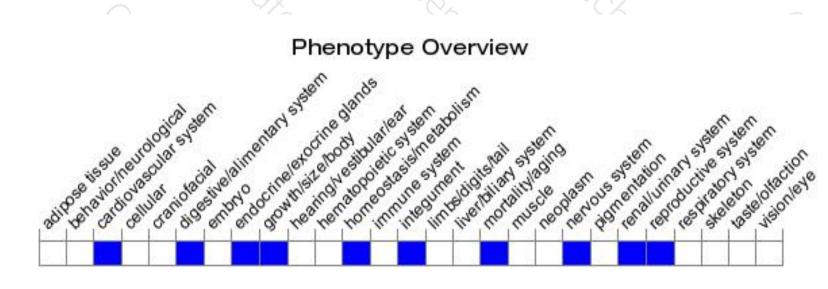
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5. ?)	ENSMUSP00000005 MobiDB lite Low complexity (Seg)					Normal Sta	× <sup>2</sup> S
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	SMART		Tetrab	ricopeptide repeat			
	<u>Pfam</u>	SGTA, homodimerisa	tion domain	Tetratricopeptide re	epeat 1		
			Tet	ratricopeptide repeat			
.%	PROSITE profiles		Tetrat	ricopeptide repeat-containin	g domain		6
	PANTHER	PTHR45831	Tetrab	ricopeptide repeat			
		PTHR45831:SF3					
	Gene3D	1.20.5.420	Tetratrico;	peptide-like helical domain s	uperfamily		
$\sum$		Sequence variants	(dbSNP and all other	sources)			6
	All sequence SNPs/i			1			
	Variant Legend	synonymous v	/ariant		-		.0
	Scale bar	0 40	80	120 160	200	240	315
	<sup>N</sup> CA				° CA		

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# Mouse phenotype description(MGI)





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

According to the existing MGI data,mice homozygous for a knock-out allele exhibit increased neonatal death, subfertility, decreased body size, increased brain, intestine and heart weight, decreased serum insulin-like growth factor I level, large penis, and increased testis descent.

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



