

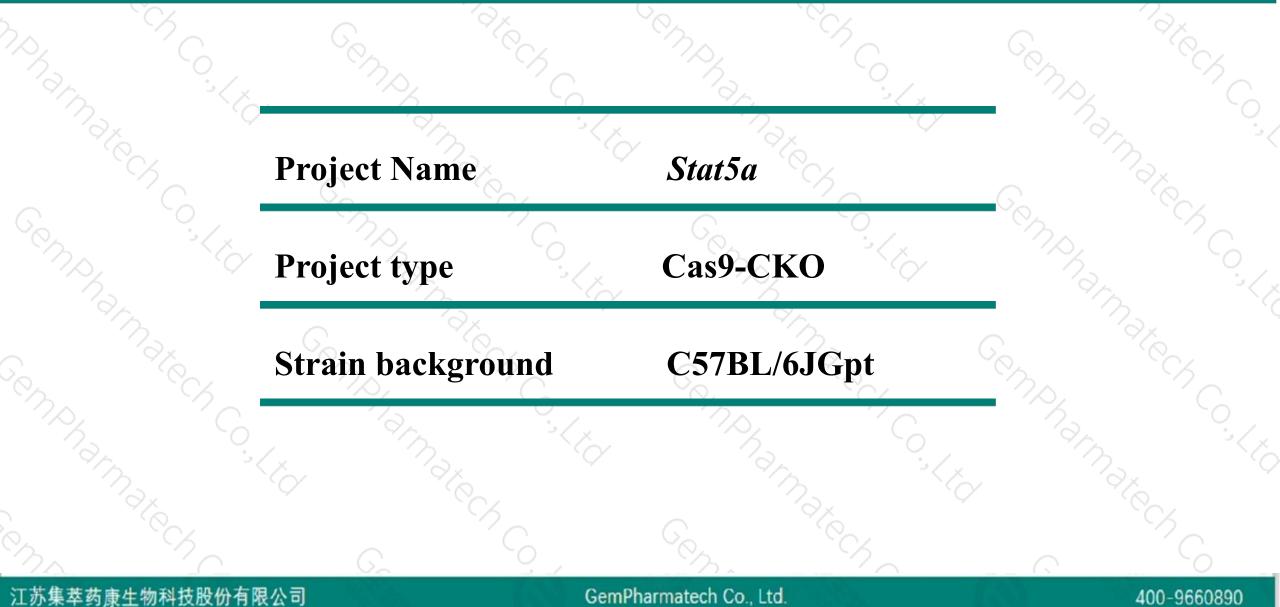
# Stat5a Cas9-CKO Strategy

Designer: Xueting Zhang Design Date: 2019-8-5

0

### **Project Overview**

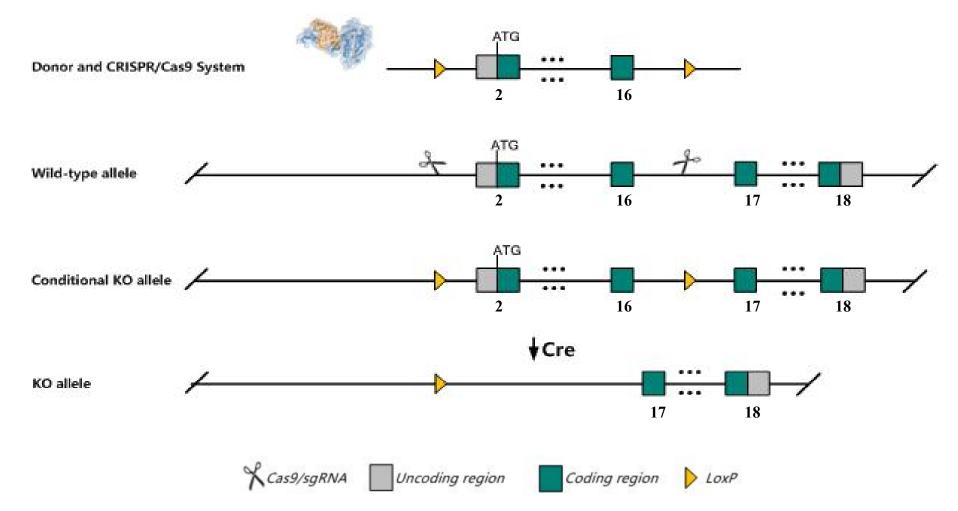




### **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Stat5a* gene. The schematic diagram is as follows:



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.



The Stat5a gene has 7 transcripts. According to the structure of Stat5a gene, exon2-exon16 of Stat5a-203 (ENSMUST00000107357.3) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Stat5a* 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 disruptions in this gene are reduced in size and display abnormalities in both mammary gland structure and function.
  - > The Stat5a gene is located on the Chr11. 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)**



\$ ?

### Stat5a signal transducer and activator of transcription 5A [Mus musculus (house mouse)]

Gene ID: 20850, updated on 25-Mar-2019

#### Summary

Official Symbol	Stat5a provided by MGI						
Official Full Name	signal transducer and activator of transcription 5A provided by MGI						
Primary source	MGI:MGI:103036						
See related	Ensembl:ENSMUSG0000004043						
Gene type	protein coding						
<b>RefSeq status</b>	VALIDATED						
Organism	Mus musculus						
Lineage							
	Muroidea; Muridae; Murinae; Mus; Mus						
Also known as	AA959963, STAT5						
Expression	Broad expression in thymus adult (RPKM 40.3), mammary gland adult (RPKM 29.6) and 20 other tissues See more						
Orthologs	human all						

#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

#### 400-9660890

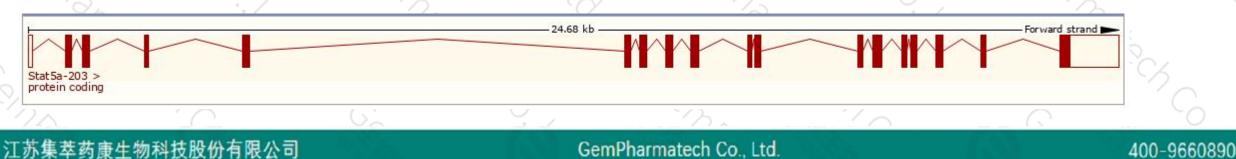
## **Transcript information (Ensembl)**



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

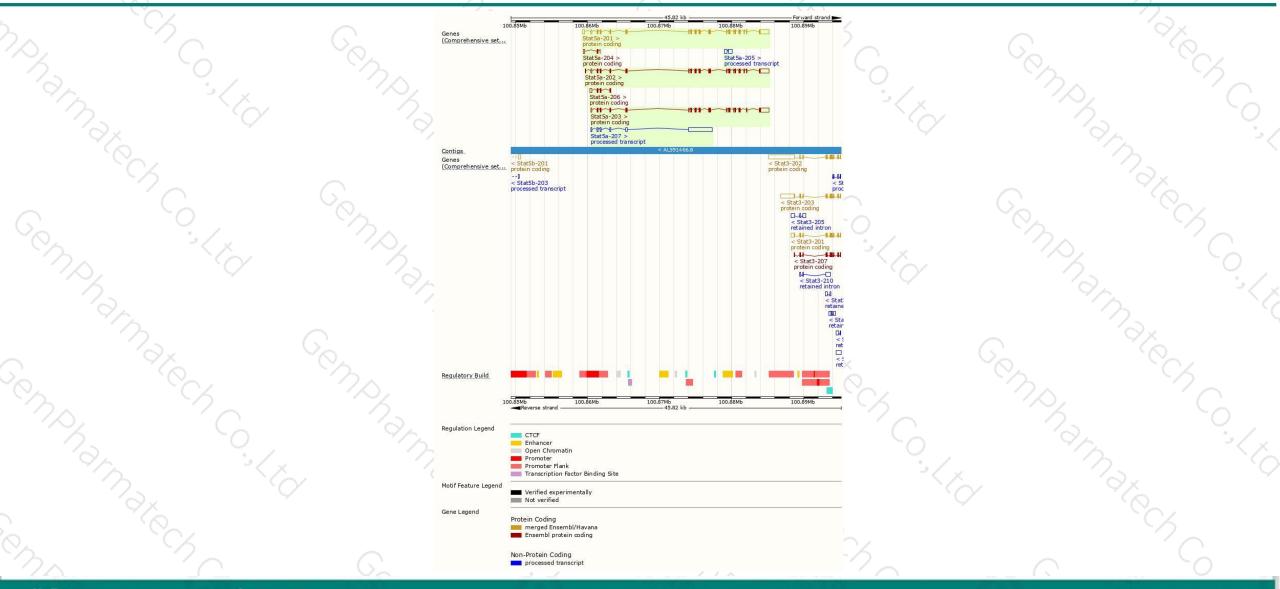
1 March	The second se	and the state		1 allow			the lines. A state
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Stat5a-201	ENSMUST0000004145.13	3888	<u>793aa</u>	Protein coding	CCDS25439	P42230 Q9JIA0	TSL:1 GENCODE basic APPRIS P1
Stat5a-202	ENSMUST00000107356.7	3738	<u>793aa</u>	Protein coding	CCDS25439	P42230 Q9JIA0	TSL:1 GENCODE basic APPRIS P1
Stat5a-203	ENSMUST00000107357.3	3605	<u>797aa</u>	Protein coding	CCDS48933	B2C3G8	TSL:1 GENCODE basic
Stat5a-206	ENSMUST00000138083.7	617	<u>125aa</u>	Protein coding	20	A2A5D3	CDS 3' incomplete TSL:2
Stat5a-204	ENSMUST00000133036.7	336	<u>51aa</u>	Protein coding	7.1	A2A5D4	CDS 3' incomplete TSL:3
Stat5a-207	ENSMUST00000154087.1	3958	No protein	Processed transcript	-	87	TSL:1
Stat5a-205	ENSMUST00000135272.1	850	No protein	Processed transcript	10	84	TSL:3

The strategy is based on the design of Stat5a-203 transcript, The transcription is shown below



### **Genomic location distribution**





江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890

### **Protein domain**

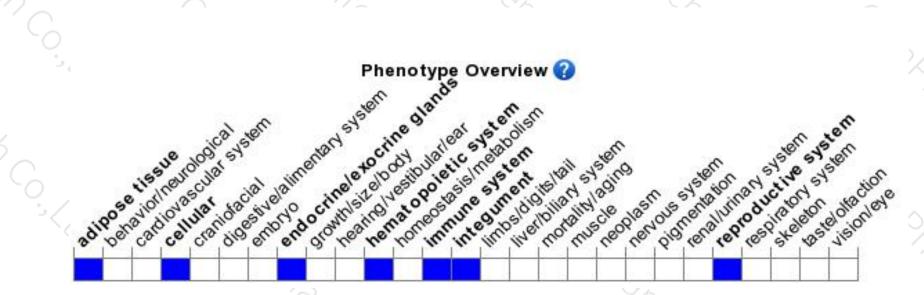


	~~~~~~			97	6	2	×2°.	~		×
	ENSMUSP00000102 Low complexity (Seg) Coiled-coils (Ncoils) Superfamily		STAT	ranscription factor, coiled	coil			SH2 domain superfa	mily	30
-	SMART	A REPORT OF A REPORT OF A REPORT	factor, N-terminal di factor, protein inter		p53-like	transcription factor, I	DNA-binding	SH2 domain	-	
	Pfam.	STAT transcription	factor, protein inter	action transcription factor, all-al		transcription factor, D	NA-binding	SH2 domain		
	PROSITE profiles PANTHER	STAT5a/5b			prie somerni,			SH2 domain		12
	Gene3D	Transcription factor	1.20.10		STAT t	ranscription factor, DN	IA-binding, N-termina	SH2 domain super	amily	1-34
	CDD		factor, N-terminal d				1.10.238.10	cd10421		Ì
	All sequence SNPs/i	Sequence vanan	ts (dbSNP and all	other sources)	00.0	e ne reno	с та	n 🖷 🕐	1.11.11	
	Variant Legend	missense v	ariant			synor	nymous variant			0
2	Scale bar	0	80	160 240	320	400	480	560 640	79	한 작품
	March			axe Ch Co			marech	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1378 C	G.
裝	药康生物科技股份	有限公司			GemPharn	natech Co., Lt	:d.		400-9	9660890

江苏集萃药康生物科技股 限公司

### 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 disruptions in this gene are reduced in size and display abnormalities in both mammary gland structure and function.

#### 江苏集萃药康生物科技股份有限公司

#### GemPharmatech Co., Ltd.

#### 400-9660890



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



