

# Mtx3 Cas9-CKO Strategy

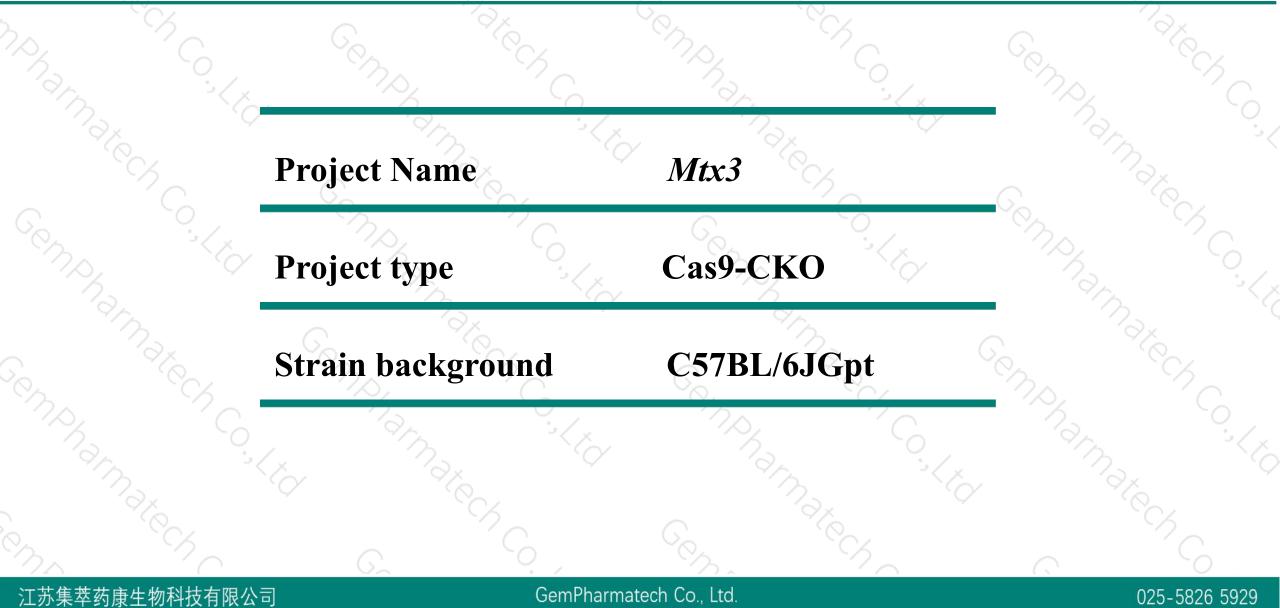
Designer: Zihe Cui

**Reviewer: Jia Yu** 

Design Date: 2020-10-15

# **Project Overview**



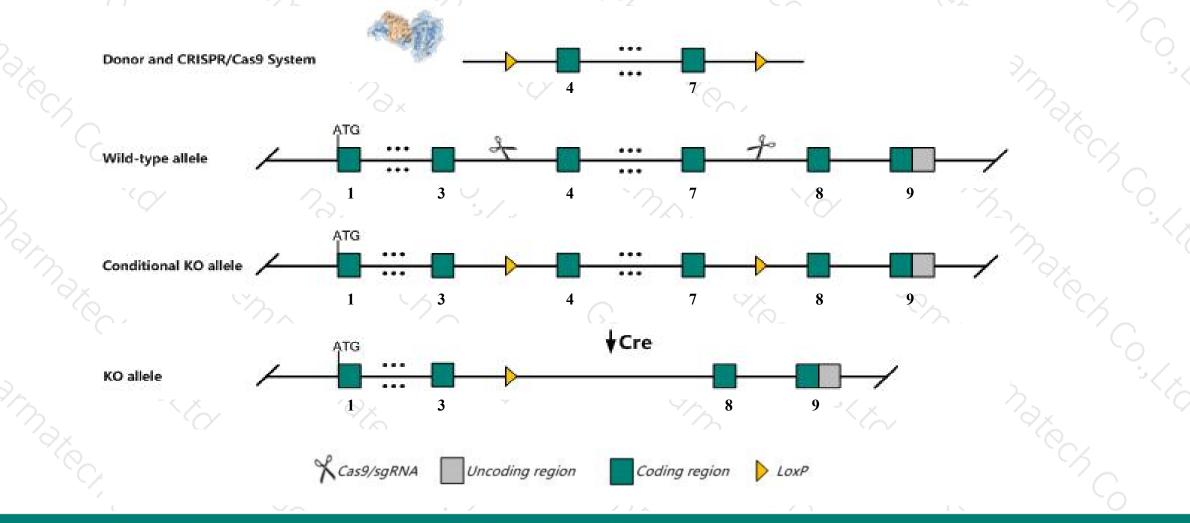


# **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the *Mtx3* gene. The schematic diagram is as follows:



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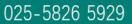
The *Mtx3* gene has 3 transcripts. According to the structure of *Mtx3* gene, exon4-exon7 of *Mtx3*-201(ENSMUST0000076169.3) transcript is recommended as the knockout region. The region contains 511bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Mtx3* 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.



- > The Mtx3 gene is located on the Chr13. 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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#### Mtx3 metaxin 3 [ Mus musculus (house mouse) ]

Gene ID: 382793, updated on 25-Sep-2020

#### Summary

Official Symbol Mtx3 provided by MGI Official Full Name metaxin 3 provided by MGI Primary source MGI:MGI:2686040 Ensembl:ENSMUSG0000021704 See related protein coding Gene type RefSeq status VALIDATED Organism Mus musculus Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Lineage Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as Gm1194; Gm6514; AA409304; Al853833; AU067765; EG624619; 4930470O13Rik Ubiquitous expression in limb E14.5 (RPKM 7.2), cerebellum adult (RPKM 6.6) and 27 other tissues See more Expression Orthologs human all

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# **Transcript information (Ensembl)**

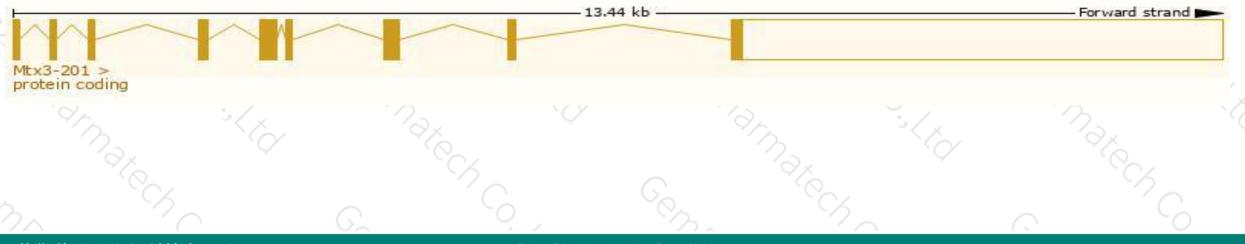


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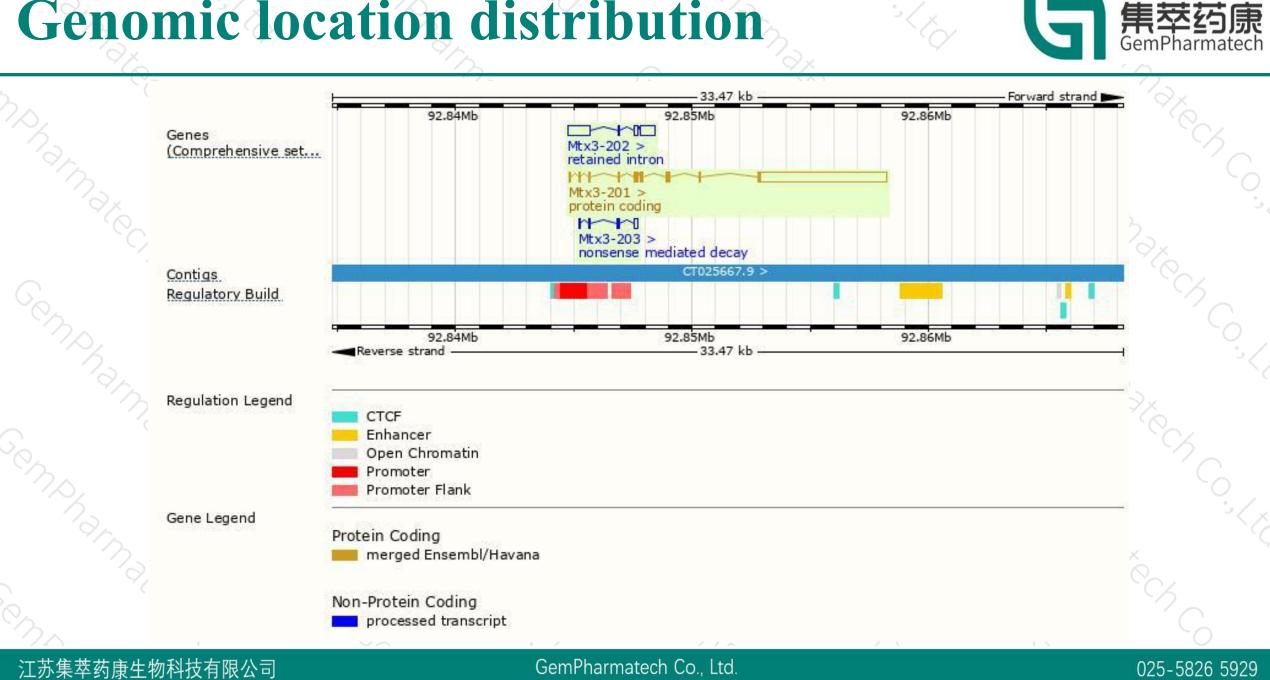
The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mtx3-201	ENSMUST0000076169.3	6275	<u>312aa</u>	Protein coding	CCDS49328	D3YTP3	TSL:5 GENCODE basic APPRIS P1
Mtx3-203	ENSMUST00000224368.1	435	<u>68aa</u>	Nonsense mediated decay	÷	A0A286YDW3	CDS 5' incomplete
Mtx3-202	ENSMUST00000223719.1	1810	No protein	Retained intron	2	6 <u>1</u> 6	

The strategy is based on the design of *Mtx3-201* transcript, the transcription is shown below:



### **Genomic location distribution**



## **Protein domain**



<u>`</u>							100
ENSMUSP00000075 Low complexity (Seg)		217		10			-
Superfamily				Glutathio	ne S-transferase,	C-terminal domain su	perfamily
SFLD	Glutathione Transfer	rase family				-	
	SFLDG01180		-14				
Pfam.	Mitochondria	al outer membrane tra	nsport complex San	(A) 22			
PIRSF					xin, glutathione S	-transferase domain	
PANTHER		rembrarie transport co	mplex protein, met:	axin			20
	PTHR12289(SF30						
Gene3D	FILMALEUS			1.20.1050	10		
CDD.	cd03078		cd03212				
All sequence SNPs/i	Sequence variants	(dbSNP and all othe	er sources)				
Variant Legend	<u>-</u>						
	missense varia						
Scale bar	<b>o</b> 40	80	120	160	200	240	312
<u> </u>	62	<u> </u>	<u>^</u> ^	A	10	R	
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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



