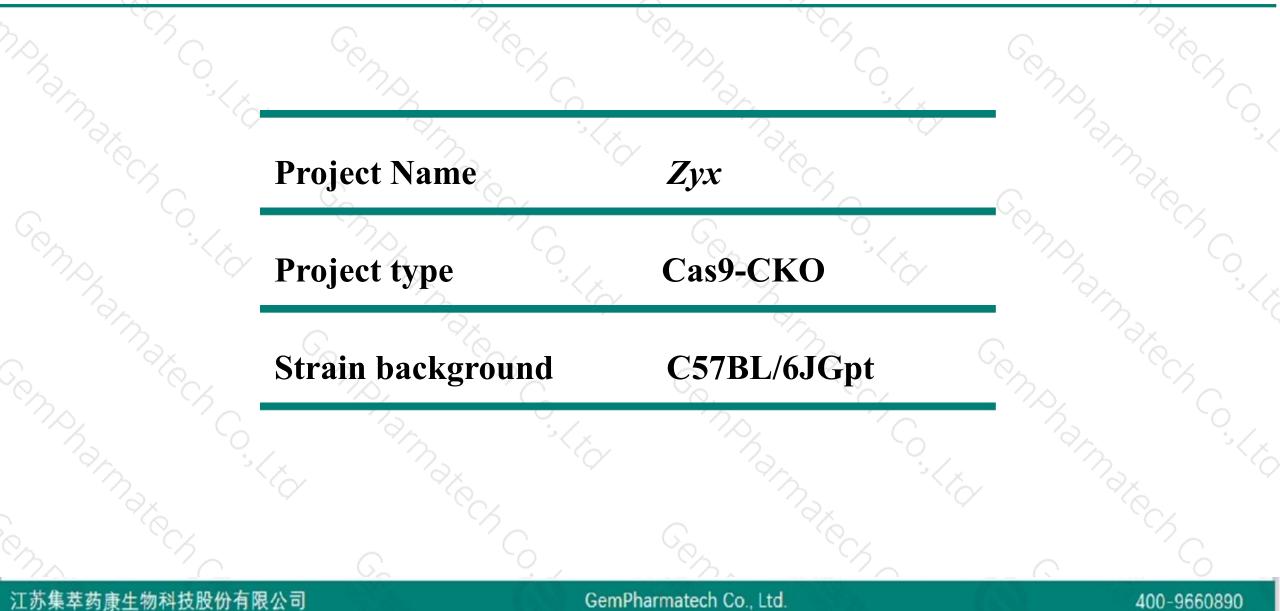


Zyx Cas9-CKO Strategy

Designer: Reviewer: Design Date: Yang Zeng Xueting Zhang 2019-10-29

Project Overview



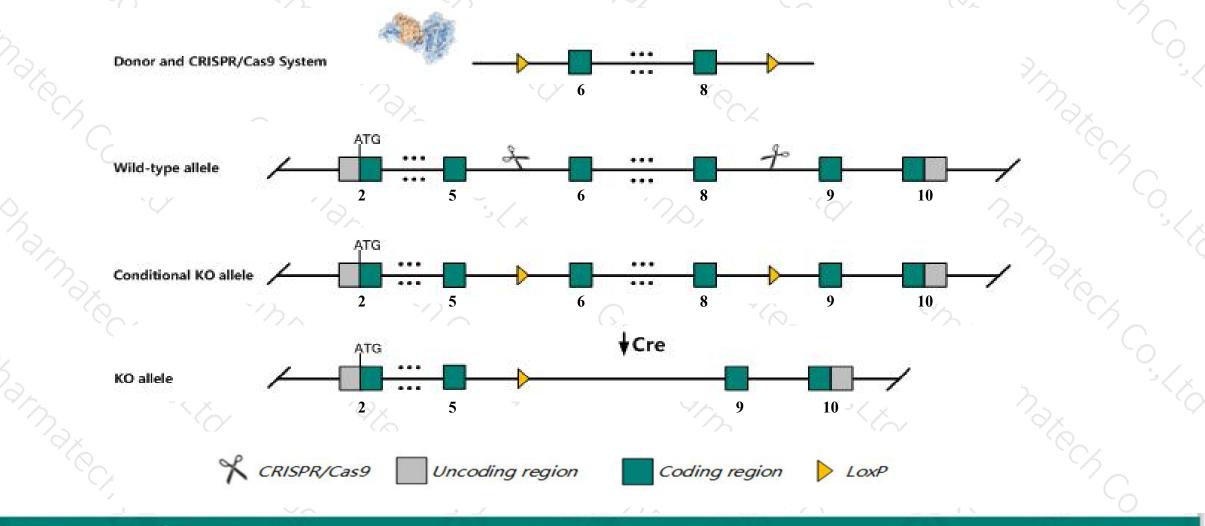


Conditional Knockout strategy



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



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The Zyx gene has 11 transcripts. According to the structure of Zyx gene, exon6-exon8 of Zyx-202 (ENSMUST00000164375.3) transcript is recommended as the knockout region. The region contains 470bp coding sequence. Knock out the region will result in disruption of protein function.

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



> According to the existing MGI data, Mice lacking functional copies of this gene are viable, fertile, and develop normally.

- Transcript Zyx-204/206/ 208/209 may not be affected. The KO region is close to Epha1 gene, Knockout the region may affect the regulatory function of Epha1 gene.
- > The Zyx gene is located on the Chr6. 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)



Zyx zyxin [Mus musculus (house mouse)]

Gene ID: 22793, updated on 3-Sep-2019

Summary

< ? (

Official Symbol	Zyx provided by MGI
Official Full Name	Zyxin provided by MGI
Primary source	MGI:MGI:103072
See related	Ensembl:ENSMUSG0000029860
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; Musi; Mus
Also known as	R75157: 9530098H06Rik

Chromosome 6 - NC_000072.6

Z9x=

2010310C07Rik=

Epho1 +

42401119

Tas2r143

so known as R/5157; 9530098H0

[42284851)

Clent

Expression Ubiquitous expression in lung adult (RPKM 88.7), ovary adult (RPKM 71.5) and 28 other tissues See more

L0C115490521 🧄

Fam131b

Orthologs human all

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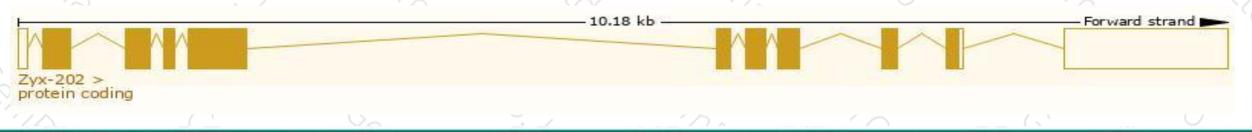
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Transcript information (Ensembl)



lame 🍦	Transcript ID	bp 🛊	Protein 🛊	Translation ID	Biotype 💧	CCDS 🖕	UniProt 🖕	Flags	
Zyx-202	ENSMUST00000164375.3	3188	<u>564aa</u>	ENSMUSP00000126622.1	Protein coding	<u>CCDS20066</u> &	<u>Q62523</u>	TSL:1 GENCODE basic	APPRIS P3
Zyx-207	ENSMUST00000203652.2	2605	<u>564aa</u>	ENSMUSP00000145451.1	Protein coding	<u>CCDS20066</u>	Q62523	TSL:1 GENCODE basic	APPRIS P3
Zyx-201	ENSMUST0000070635.12	2301	<u>533aa</u>	ENSMUSP0000070427.7	Protein coding	CCDS85034	<u>Q7TQE2</u> @	TSL:1 GENCODE basic	APPRIS ALT
Zyx-205	ENSMUST00000203401.2	1954	<u>533aa</u>	ENSMUSP00000145236.1	Protein coding	<u>CCDS85034</u>	Q7TQE2	TSL:1 GENCODE basic	APPRIS ALT
Zyx-208	ENSMUST00000203846.2	695	<u>212aa</u>	ENSMUSP00000145189.1	Protein coding	10	A0A0N4SVP4	CDS 3' incomplete	TSL:3
Zyx-209	ENSMUST00000203849.1	525	<u>109aa</u>	ENSMUSP00000145052.1	Protein coding	-	A0A0N4SVD2	CDS 3' incomplete	TSL:2
Zyx-204	ENSMUST00000203329.2	499	<u>137aa</u>	ENSMUSP00000144863.1	Protein coding	85	<u>A0A0N4SUX7</u> &	CDS 3' incomplete	TSL:3
Zyx-203	ENSMUST00000203290.2	3076	No protein	-	Retained intron	-	1949	TSL:2	
yx-206	ENSMUST00000203626.1	729	No protein	5	IncRNA	85	0.70	TSL:2	
yx-211	ENSMUST00000204480.1	351	No protein	-	IncRNA	-	1949	TSL:3	
Zyx-210	ENSMUST00000204270.2	344	No protein	- 	IncRNA	-	0.70	TSL:2	

The strategy is based on the design of Zyx-202 transcript, The transcription is shown below



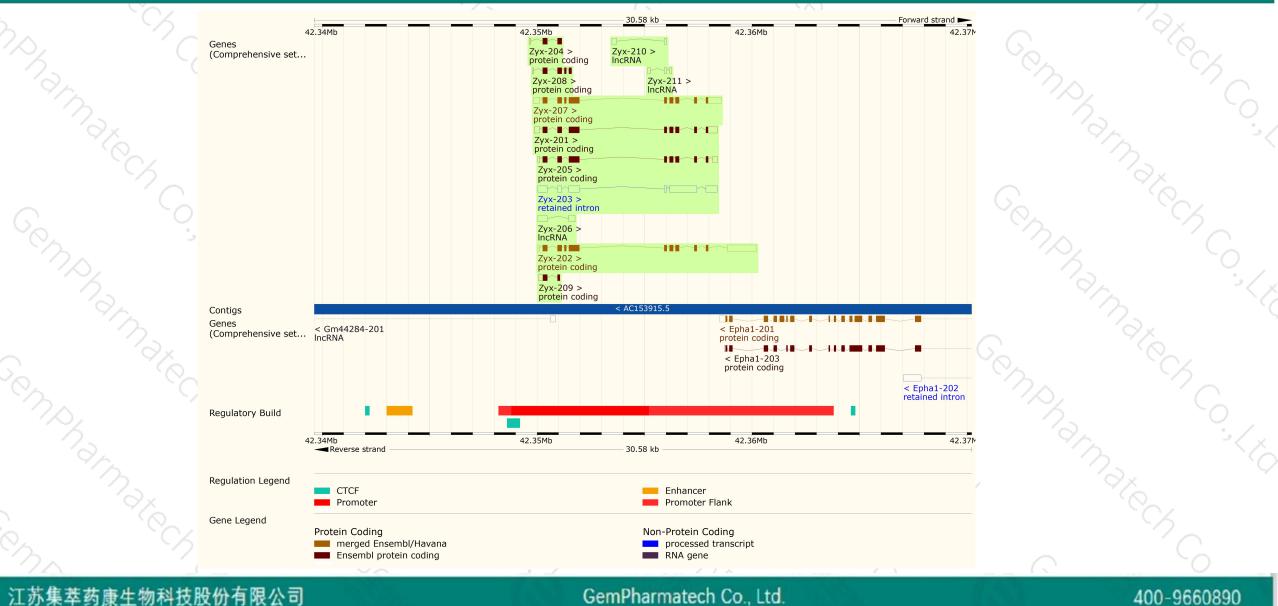
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Genomic location distribution





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Protein domain



	ENSMUSP00000126 MobiDB lite		ns,		~^	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	2	0		no.		30
Ż	Low complexity (Seg) Superfamily SMART							Zinc finger, L				0
	Pfam PROSITE profiles PROSITE patterns							Zinc finger, I Zinc finger, L Zinc finger, I	.IM-type		?	
	PANTHER	PTHR24212										·2~
	Gene3D CDD	PTHR24212:SF1						2.10.110.10	cd09353	cd09435		0
									C009355	009455		~ ~ ~
25	All sequence SNPs/i	<u> </u>	(dbSNP and all other	· sources)	1	1 1 1			C009555			~ <
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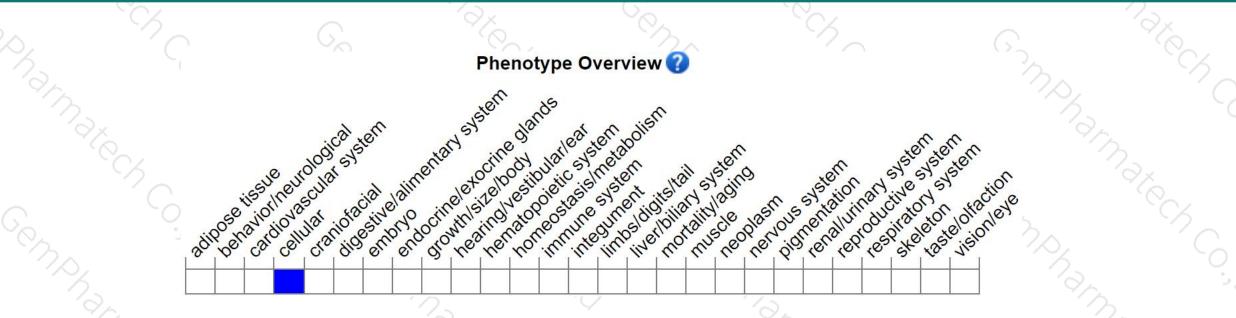
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Mouse phenotype description(MGI)





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 lacking functional copies of this gene are viable, fertile, and develop normally.



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



