

Zhx2 Cas9-CKO Strategy

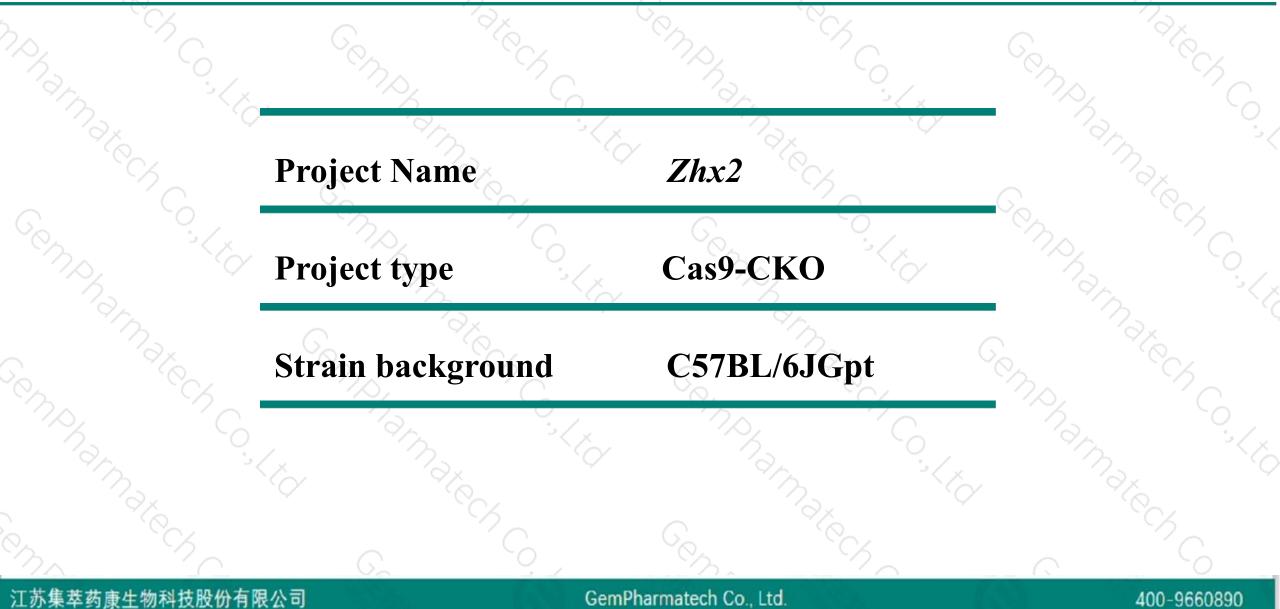
Designer: Reviewer:

Design Date:

Huan Fan Huan Wang 2020-2-28

Project Overview

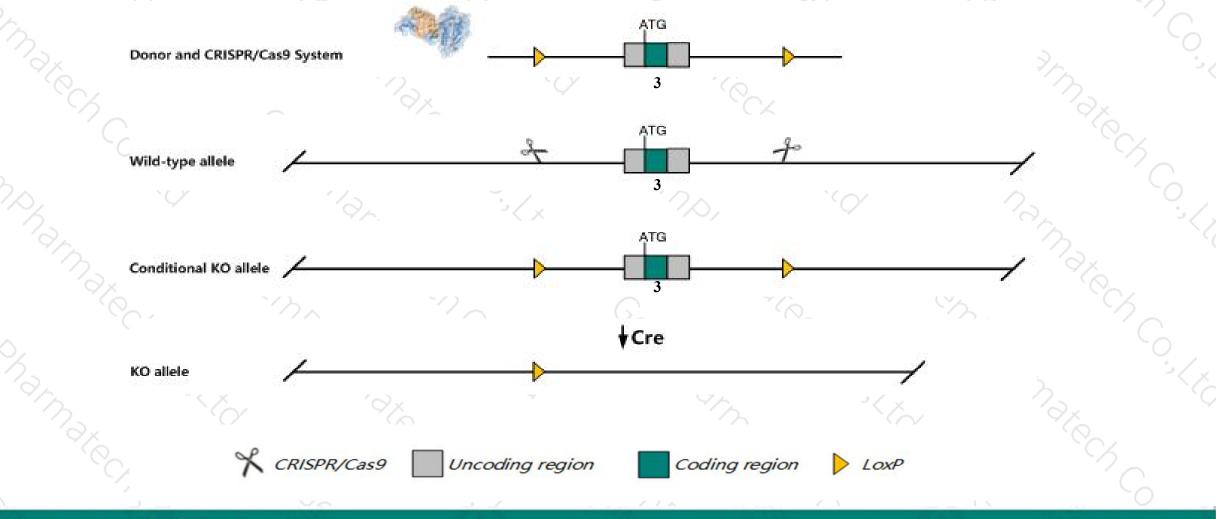




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Zhx2* gene. The schematic diagram is as follows:



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The Zhx2 gene has 2 transcripts. According to the structure of Zhx2 gene, exon3 of Zhx2-201 (ENSMUST00000096430.10) transcript is recommended as the knockout region. The region contains all 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 *Zhx2* 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, Alpha-fetoprotein levels in plasma decline precipitously after birth. This gene regulates a difference in adult level and rate of neonatal decrease of AFP. The BALB/cJGpt substrain carries a genetic variant allele determining a slow rate of decline to adultlevel.
- The Zhx2 gene is located on the Chr15. 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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Zhx2 zinc fingers and homeoboxes 2 [Mus musculus (house mouse)]

Gene ID: 387609, updated on 2-Apr-2019

Summary

Official Symbol	Zhx2 provided by MGI
Official Full Name	zinc fingers and homeoboxes 2 provided by MGI
Primary source	MGI:MGI:2683087
See related	Ensembl:ENSMUSG00000071757
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; Murinae; Mus; Mus
Also known as	Afr-1, Afr1, Raf, mKIAA0854
Expression	Ubiquitous expression in genital fat pad adult (RPKM 5.9), spleen adult (RPKM 5.8) and 28 other tissues See more
Orthologs	human all

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The gene has 2 transcripts, all transcripts are shown below:

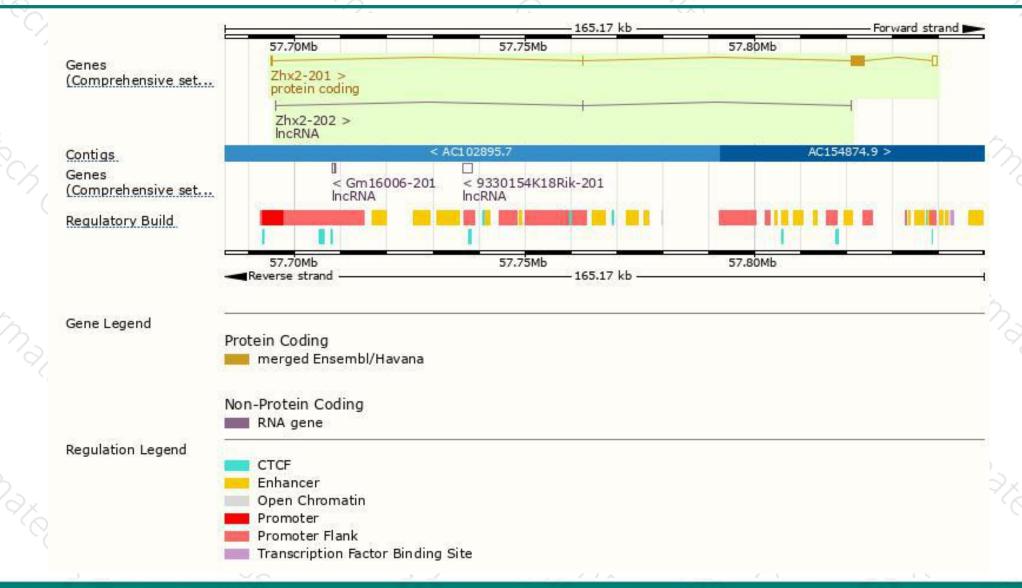
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zhx2-201	ENSMUST0000096430.10	4376	<u>836aa</u>	Protein coding	CCDS27483	<u>Q8C0C0</u>	TSL:1 GENCODE basic APPRIS P1
Zhx2-202	ENSMUST00000160990.1	211	No protein	IncRNA	(8.	TSL:1

The strategy is based on the design of *Zhx2-201* transcript, The transcription is shown below

			145.:	17 kb	For	ward strand	
Zhx2-201 > protein coding	1		1				n Haller
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Genomic location distribution





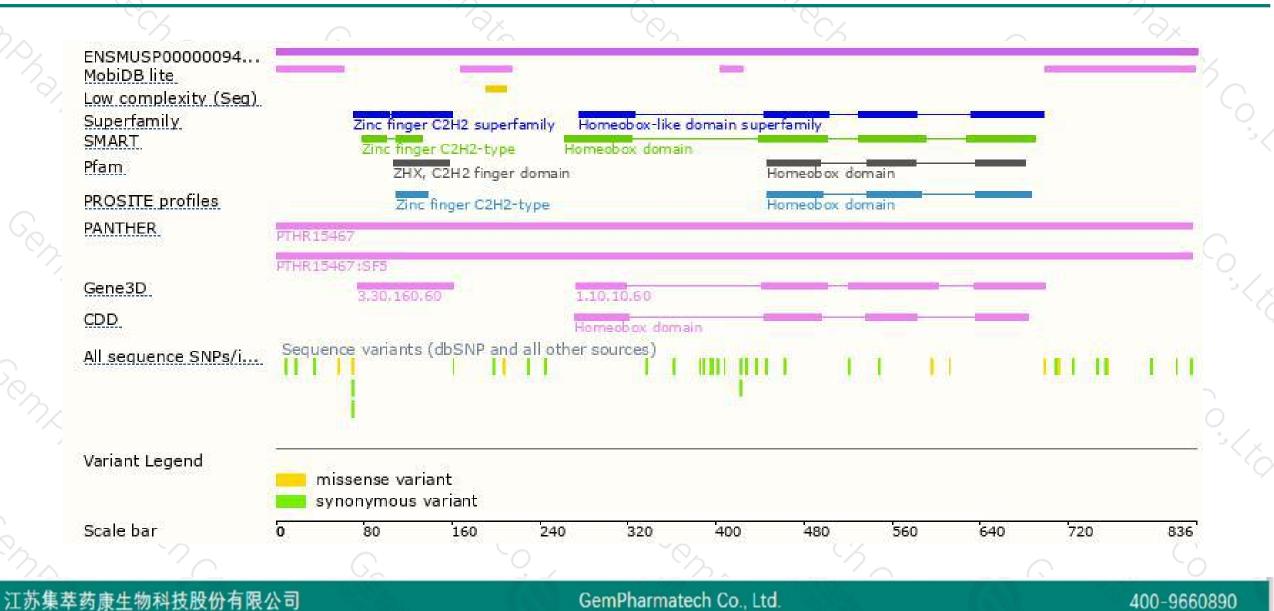
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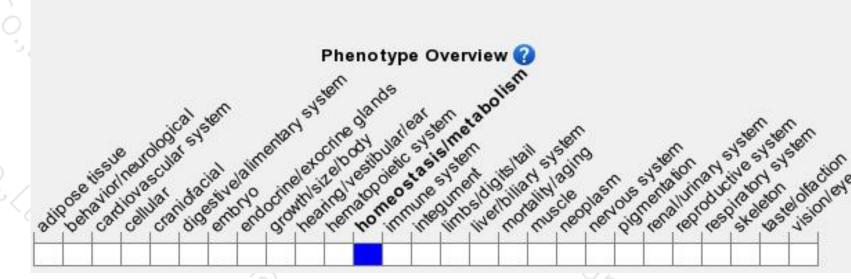
Protein domain





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, Alpha-fetoprotein levels in plasma decline precipitously after birth. This gene regulates a difference in adult level and rate of neonatal decrease of AFP. The BALB/cJGpt substrain carries a genetic variant allele determining a slow rate of decline to adultlevel.

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



