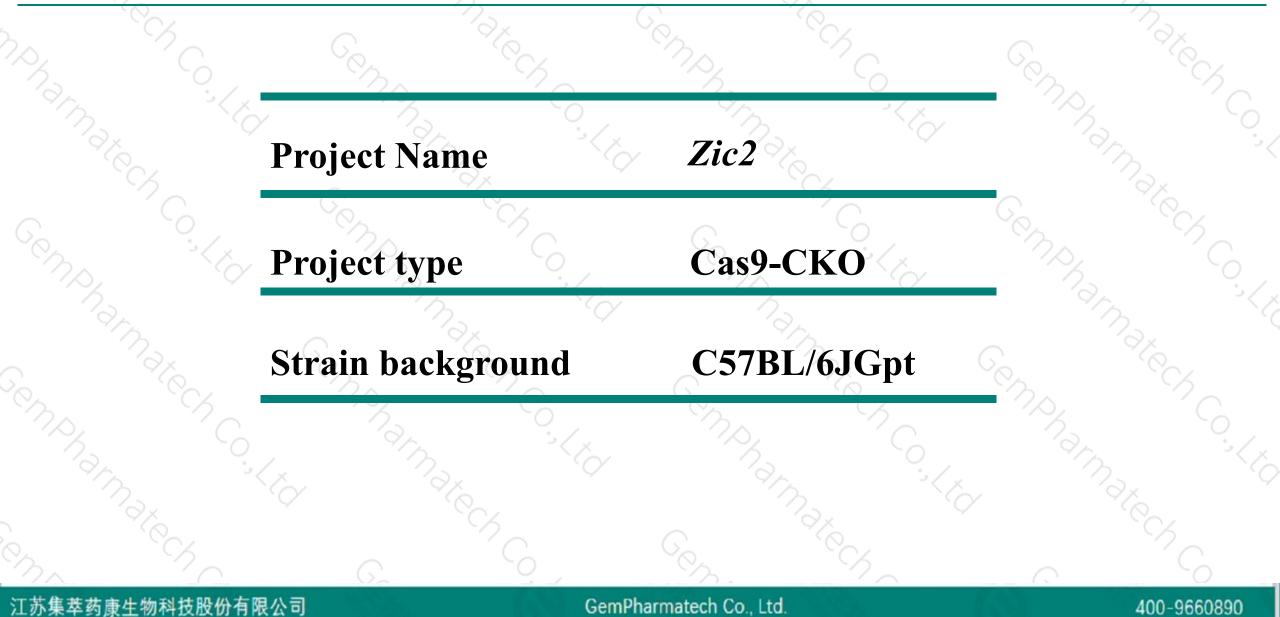
# Zic2 Cas9-CKO Strategy

harman

Designer: Huan Fan Design Date: 2019-8-4

## **Project Overview**

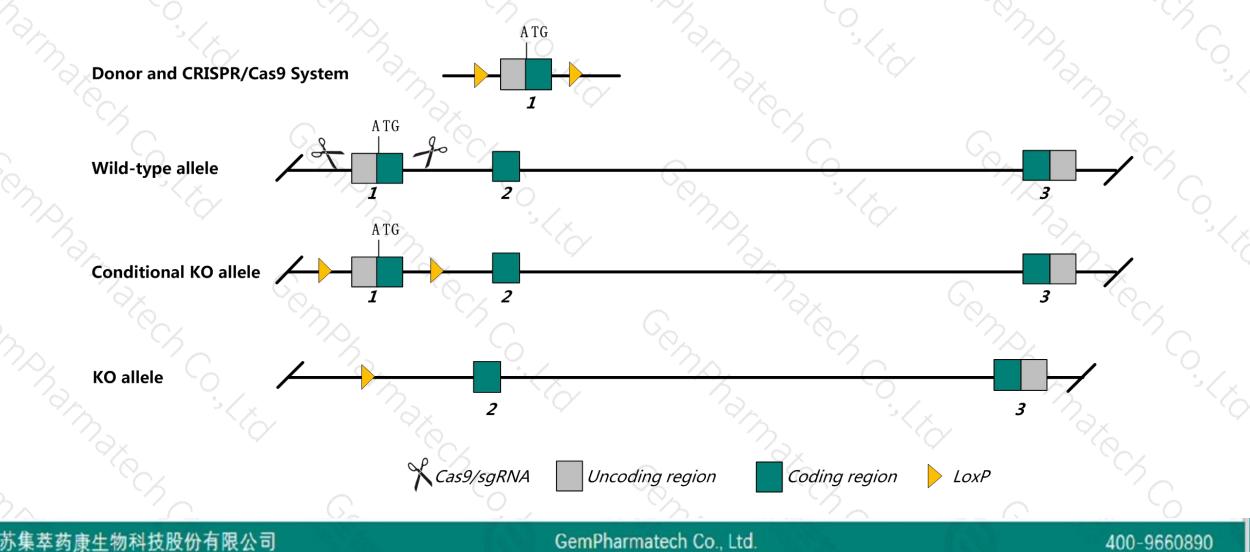




### **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Zic2 gene. The schematic diagram is as follows:





- The Zic2 gene has 2 transcripts. According to the structure of Zic2 gene, exon1 of Zic2-201(ENSMUST00000075888.5) transcript is recommended as the knockout region. The region contains start codon ATG of coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Zic2* 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 or cell types.

### Notice



- According to the existing MGI data, Defects in neurulation and forebrain development have been identified in both targeted and ENU induced homozygous mutants. Death occurs perinatally in the targeted mouse and during midgestation in the ENU mouse. Mice homozygous for a knock-down allele exhibit cognitive and social behavior defects.
- The KO region contains functional region of the 2610035F20Rik gene.Knockout the region may affect the function of 2610035F20Rik gene.
- The Zic2 gene is located on the Chr14. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

## Gene information (NCBI)



\$ ?

Zic2 zinc finger protein of the cerebellum 2 [ Mus musculus (house mouse) ]

Gene ID: 22772, updated on 23-Apr-2019

#### Summary

Official Symbol Zic2 provided by MGI Official Full Name zinc finger protein of the cerebellum 2 provided by MGI MGI:MGI:106679 Primary source See related Ensembl:ENSMUSG0000061524 Gene type protein coding RefSeg 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 Ku: HPE5 Expression Biased expression in cerebellum adult (RPKM 37.4), whole brain E14.5 (RPKM 15.2) and 5 other tissues See more Orthologs human all

## **Transcript information (Ensembl)**



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

Show/hide columns (1 hidden)									
Name 🖕	Transcript ID	bp 🍦	Protein 🛔	Biotype 💧	CCDS 🖕	UniProt 🝦	Flags		+
Zic2-201	ENSMUST0000075888.5	2440	<u>529aa</u>	Protein coding	<u>CCDS27349</u> &	F8VPV3团	TSL:1	GENCODE basic	APPRIS P1
Zic2-202	ENSMUST00000137059.1	765	No protein	Retained intron	<u>1</u> 8	<u></u>		TSL:2	

The strategy is based on the design of Zic2-201 transcript, The transcription is shown below

4.42 kb

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Zic2-201 > protein coding

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Forward strand

### **Genomic location distribution**



	122.466Mb 12	2.468Mb 122.470M	122.472 Mb	122.474Mb	122.476Mb	24.42 kb	122.480 Mb	122.482Mb	122.484Mb	122.486 Mb	122.4
Genes (Comprehensive set	122,400 MD 12	2.466 MD 122.470 M	) 122.472MD	122,474100	Zic2-201 > protein coding			122,462 MD	122,464 (10)	122.406100	122.4
Contigs Genes (Comprehensive set	□ < Zic5-201 protein coding	re E	2610035F20Rik-201 tained intron 2610035F20Rik-202			retained ACI 54683.2					
Regulatory Build	122,466Mb 12 Reverse strand –		ncRNA	122.474Mb	122.476Mb	122.478Mb 24.42 kb	122.480Mb	122.482Mb	122.484 Mb	122.486 Mb	

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### **Protein domain**



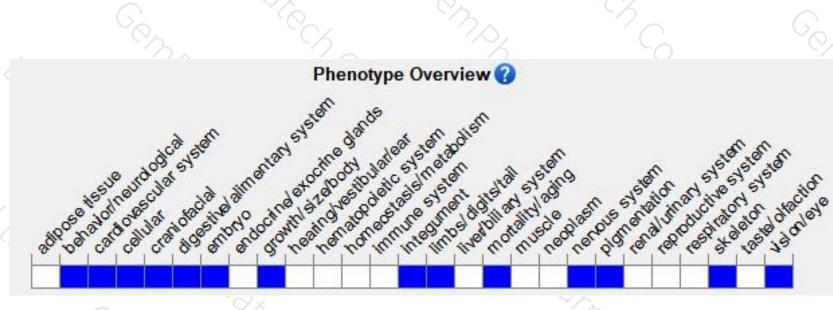
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narmax							

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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, Mutations in this locus affect cell-cycle regulation and apoptos is. Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.

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



