

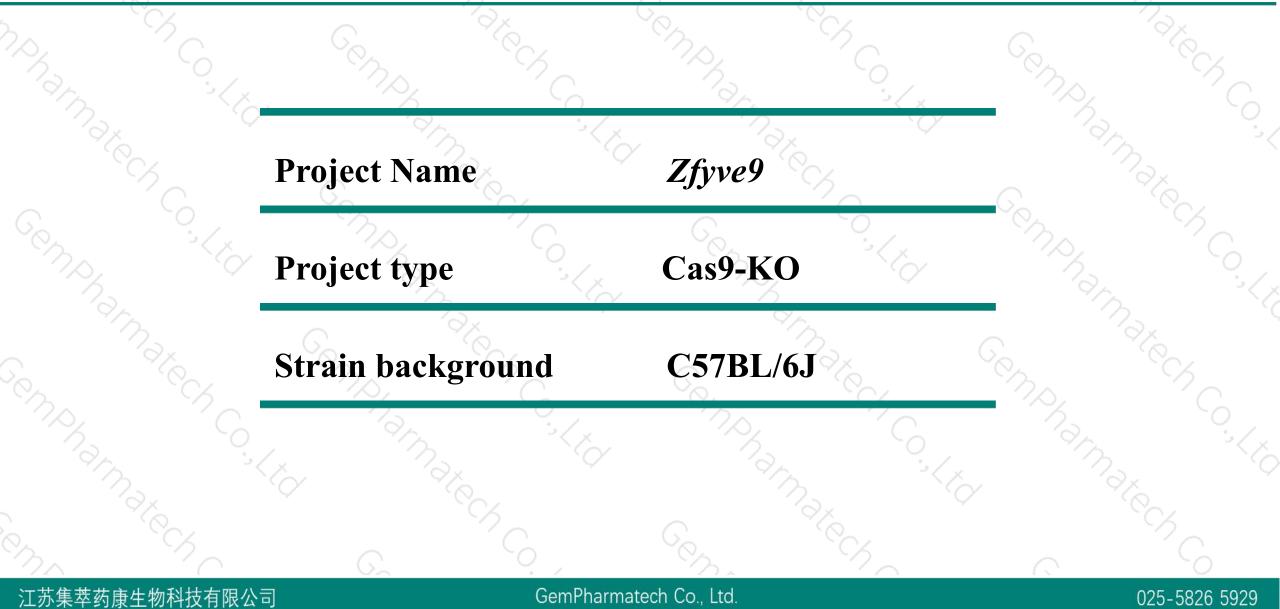
# Cemphamaten Zfyve9 Cas9-KO Strategy Cempharmareck Andraker Contra

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### **Project Overview**

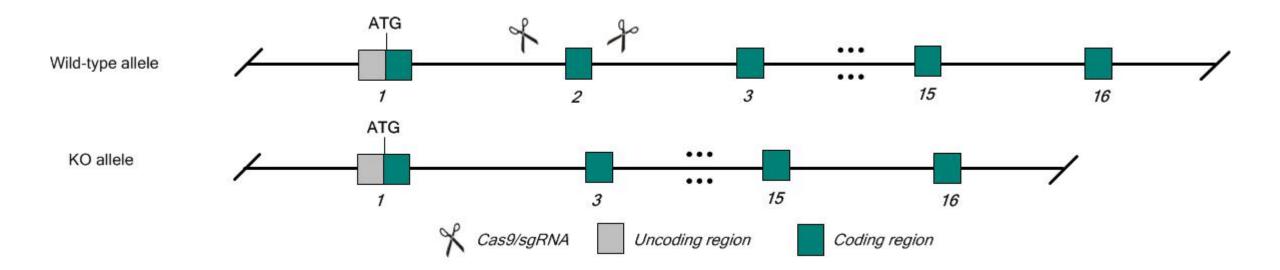




### **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Zfyve9 gene. The schematic diagram is as follows:





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- The Zfyve9 gene has 3 transcripts. According to the structure of Zfyve9 gene, exon2 of Zfyve9-201 (ENSMUST00000042185.7) transcript is recommended as the knockout region. The region contains 100bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify Zfyve9 gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

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- The Zfyve9 gene is located on the Chr4. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Notice

# **Gene information (NCBI)**



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### Zfyve9 zinc finger, FYVE domain containing 9 [Mus musculus (house mouse)]

Gene ID: 230597, updated on 31-Jan-2019

#### Summary

Official Symbol	Zfauc0						
Official Symbol	Zfyve9 provided by MGI						
Official Full Name	zinc finger, FYVE domain containing 9 provided by MGI						
Primary source	MGI:MGI:2652838						
See related	Ensembl:ENSMUSG00000034557						
Gene type	protein coding						
<b>RefSeq status</b>	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	Madhip, NSP, SARA, SMADIP						
Expression	Broad expression in cerebellum adult (RPKM 7.6), cortex adult (RPKM 4.3) and 24 other tissues See more						
Orthologs	human all						

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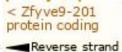
# **Transcript information (Ensembl)**



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zfyve9-202	ENSMUST00000106657.7	6459	<u>1397aa</u>	Protein coding	CCDS84772	A2A8R0	TSL:5 GENCODE basic APPRIS P2
Zfyve9-201	ENSMUST00000042185.7	2170	<u>706aa</u>	Protein coding	CCDS38834	A0S860	TSL:1 GENCODE basic
Zfyve9-203	ENSMUST00000106658.7	5987	<u>1338aa</u>	Protein coding	-	<u>A8Y5G5</u>	TSL:5 GENCODE basic APPRIS ALT2

The strategy is based on the design of Zfyve9-201 transcript, The transcription is shown below



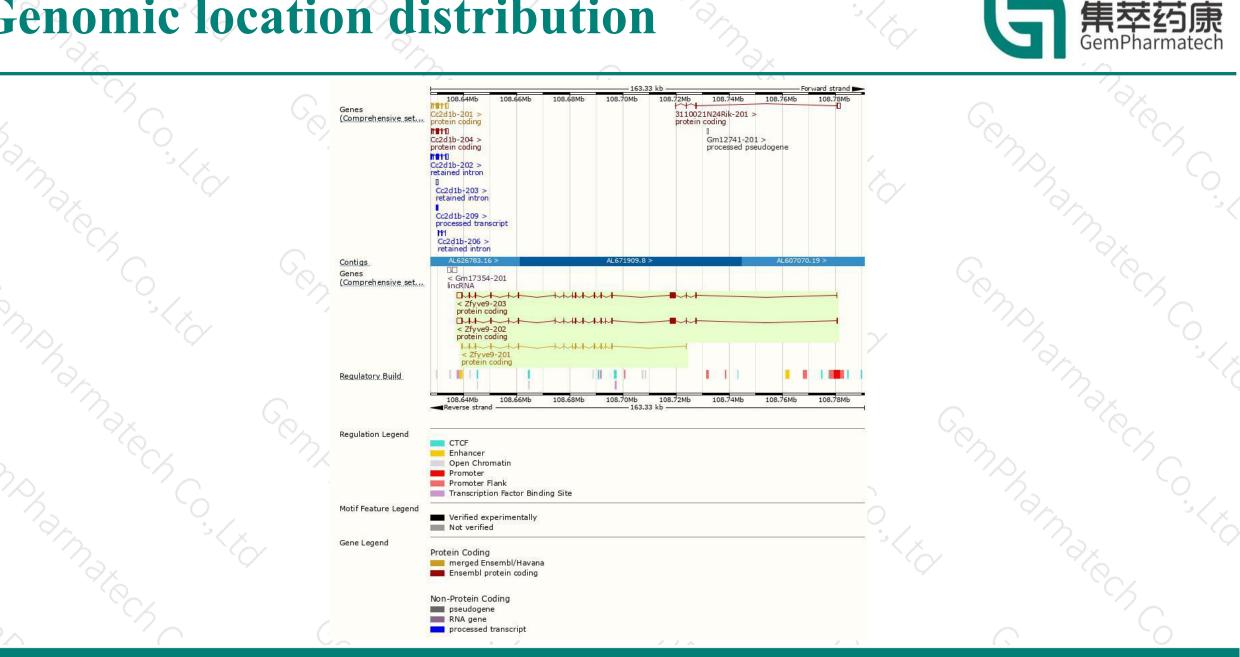
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### **Genomic location distribution**



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### **Protein domain**



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N.	ENSMUSP00000039 Low complexity (Seg) hmmpanther	PTHR22835					
	Superfamily domains Pfam domain	PTHR22835:SF265 Zinc finger, FYVE/PHD-type Smad anchor for rec	eptor activation, Smad-binding	domain Domain of unkn	own function DUF3480		
	Gene3D	Smad anchor for rec	eptor activation, Smad-binding o	Jomain superfamily	3.30.1360.220	3.30.500.40	
	All sequence SNPs/i Variant Legend	Sequence variants (dbSNP an	d all other sources)	I I I	ce region variant	TOT OF CL	1
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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



