

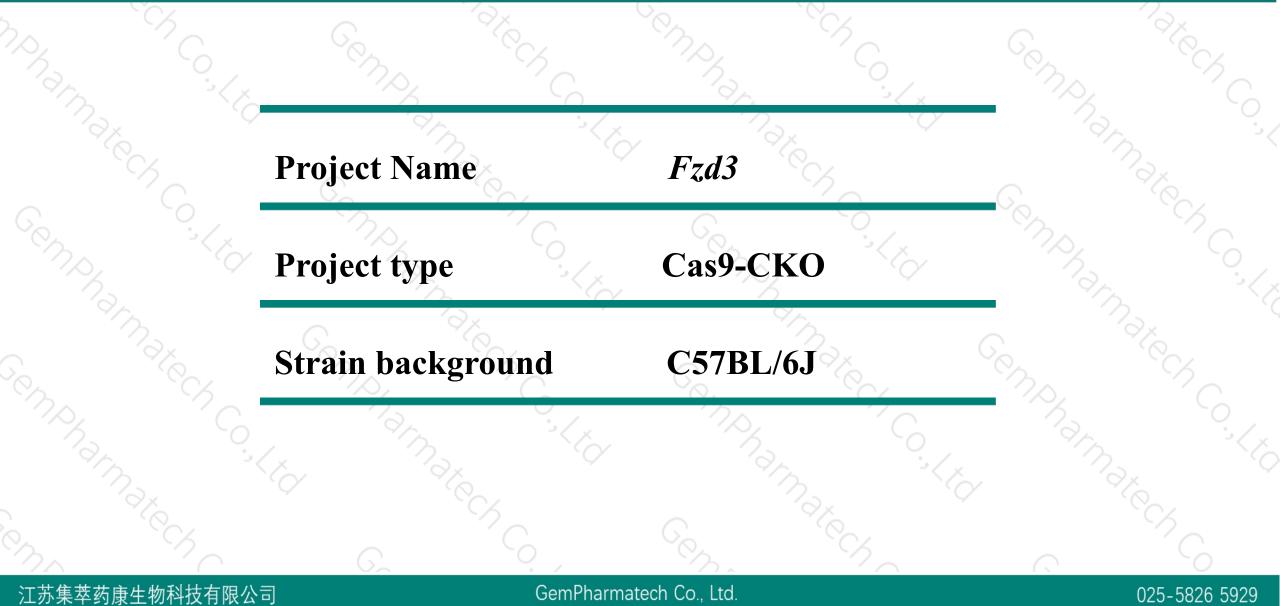
Conpharmatech Co. NDAMAK GGG-SKA Fzd3 Cas9-CKO Strategy Conplanated Co-ty

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Project Overview



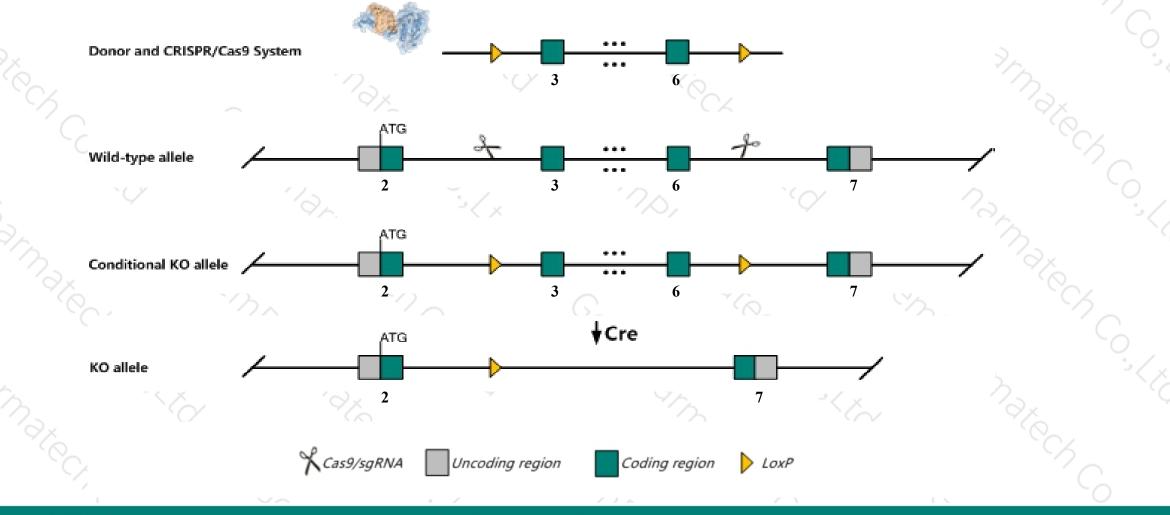


Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the *Fzd3* gene. The schematic diagram is as follows:



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The Fzd3 gene has 2 transcripts. According to the structure of Fzd3 gene, exon3-exon6 of Fzd3-202 (ENSMUST00000131309.2) transcript is recommended as the knockout region. The region contains 1598bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Fzd3* 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/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.

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.



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- According to the existing MGI data, Mice homozygous for disruption of this gene die within 30 minutes of birth. Breathing is irregular. Brain development is abnormal with occasion falure of the cephalic neural tube to close.
- The *Fzd3* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)



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Fzd3 frizzled class receptor 3 [Mus musculus (house mouse)]

Gene ID: 14365, updated on 19-Mar-2019

Summary

Official Symbol	Fzd3 provided by MGI
Official Full Name	frizzled class receptor 3 provided by <u>MGI</u>
Primary source	MGI:MGI:108476
See related	Ensembl:ENSMUSG0000007989
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	AU020229, D930050A07Rik, Fz3
Expression	Broad expression in whole brain E14.5 (RPKM 1.9), CNS E11.5 (RPKM 1.8) and 17 other tissuesSee 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		
Fzd3-202	ENSMUST00000131309.2	12734	<u>666aa</u>	Protein coding	CCDS27212	<u>Q61086</u>	TSL:1 GENCODE basic APPRIS P1		
Fzd3-201	ENSMUST00000127272.1	426	No protein	Processed transcript	-	-	TSL:3		

The strategy is based on the design of Fzd3-202 transcript, The transcription is shown below

< Fzd3-202 protein coding

Reverse strand

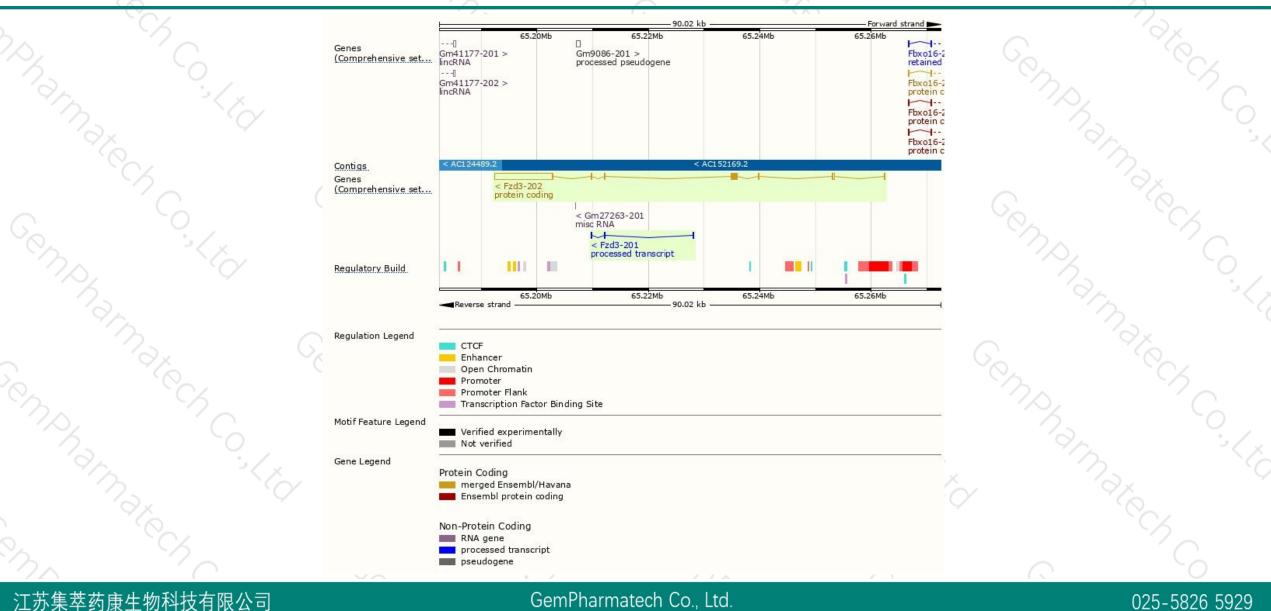
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Genomic location distribution





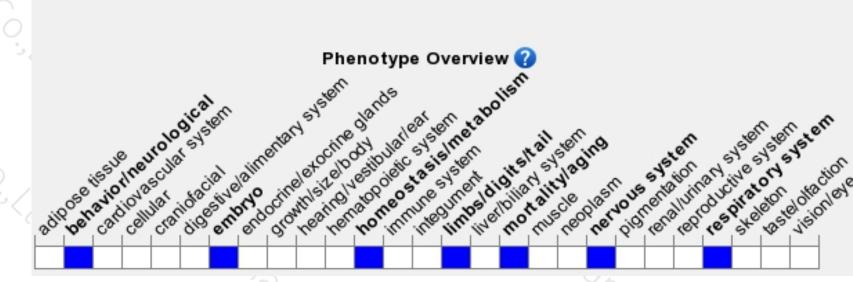
Protein domain



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	ENSMUSP00000115	-			1.								

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 homozygous for disruption of this gene die within 30 minutes of birth. Breathing is irregular. Brain development is abnormal with occasion falure of the cephalic neural tube to close.

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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



