

Zfp365 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Ruirui Zhang

Design Date: 2023-10-30

Overview

Target Gene Name

• Zfp365

Project Type

• Cas9-CKO

Genetic Background

• C57BL/6JGpt





Schematic representation of CRISPR-Cas9 engineering used to edit the Zfp365 gene.

GemPharmatech™

Technical Information

- The *Zfp365* gene has 4 transcripts. According to the structure of *Zfp365* gene, exon2 of *Zfp365*-201 (ENSMUST0000064656.8) transcript is recommended as the knockout region. The region contains start codon ATG. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Zfp365* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

GemPharmatech

Gene Information

Zfp365 zinc finger protein 365 [Mus musculus (house mouse)]

Gene ID: 216049, updated on 31-Jan-2019

Summary

Official SymbolZfp365 provided by MGIOfficial Full Namezinc finger protein 365 provided by MGIPrimary soureMGI:MGI:2143676See relatedEnsembl:ENSMUSG00000037855Gene typeprotein codingprotein codingVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso known asAl839779, AV340892, DBZ, Su48, Znf365, talaninExpressionBiased expression in cortex adult (RPKM 80.2), frontal lobe adult (RPKM 65.7) and 1 other tissueSee more
human all

2?

Source: https://www.ncbi.nlm.nih.gov/



Transcript Information

emPharmatech

The gene has 4 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zfp365-201	ENSMUST0000064656.7	4259	<u>408aa</u>	Protein coding	CCDS23903	<u>Q8BG89</u>	TSL:1 GENCODE basic APPRIS P1
Zfp365-204	ENSMUST00000138543.1	1745	No protein	Retained intron	-	*	TSL:1
Zfp365-203	ENSMUST00000132870.7	3276	No protein	IncRNA	-	2	TSL:1
Zfp365-202	ENSMUST00000127869.1	292	No protein	IncRNA	1 1 <u>4</u> 1	2	TSL:3

The strategy is based on the design of Zfp365-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org

Genomic Information



GemPharmatech

Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

GemPharmatech

Mouse Phenotype Information (MGI)



• Mice homozygous for a knock-out allele exhibit abnormal cortical basket cells in the somatosensory cortices, delayed myelination in the corpus callosum during the early postnatal period, and an increase in immature oligodendrocytes.

Source: https://www.informatics.jax.org

emPharmatech

Important Information

- According to the existing MGI data,mice homozygous for a knock-out allele exhibit abnormal cortical basket cells in the somatosensory cortices, delayed myelination in the corpus callosum during the early postnatal period, and an increase in immature oligodendrocytes.
- *Zfp365* is located on Chr10. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.



Reference

GemPharmatech[™]

Koyama Y, et al., DBZ (DISC1-binding zinc finger protein)-deficient mice display abnormalities in basket cells in the somatosensory cortices. J Chem Neuroanat. 2013 Nov;53:1-10



Fig. 2. Expression of DBZ in the developing brain and adult cerebral cortex. (A) In situ hybridization of DBZ mRNA in sagittal adult mouse brain sections at lower magnification. Scale bar, 5 mm. (B-C) In situ hybridization of DBZ mRNA in coronal brain sections at E14.5. DBZ mRNA is expressed both in the medial ganglionic eminence (MGE) and the lateral ganglionic eminence (LGE) (B), but not in the caudal ganglionic eminence (CGE) (C). MGE, medial ganglionic eminence; LGE, lateral ganglionic eminence, CGE; caudal ganglionic eminence, d, dorsal area of the MGE; and v, ventral area of the MGE. Scale bar, 200 µm. (D, F, H) In situ hybridization of DBZ (D and E) and GAD67 (F and G) mRNAs in mirror image sections of the adult mouse cortex. Arrowheads indicate neurons expressing both DBZ and GAD67 mRNAs. Sections were hybridized with a DIG-labeled sense RNA probe. (H,I) The photographs of mirror image sections are overlapping. (E, G, and I) High-magnification images of the upper panels are shown in the lower panels. Arrowheads indicate DBZ/GAD67-coexpressing neurons. Scale bars, 100 µm.

в