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Zfp365 Cas9-KO Strategy

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Reviewer:Ruirui Zhang

Design Date: 2023-10-30

Overview

Target Gene Name

- Zfp365

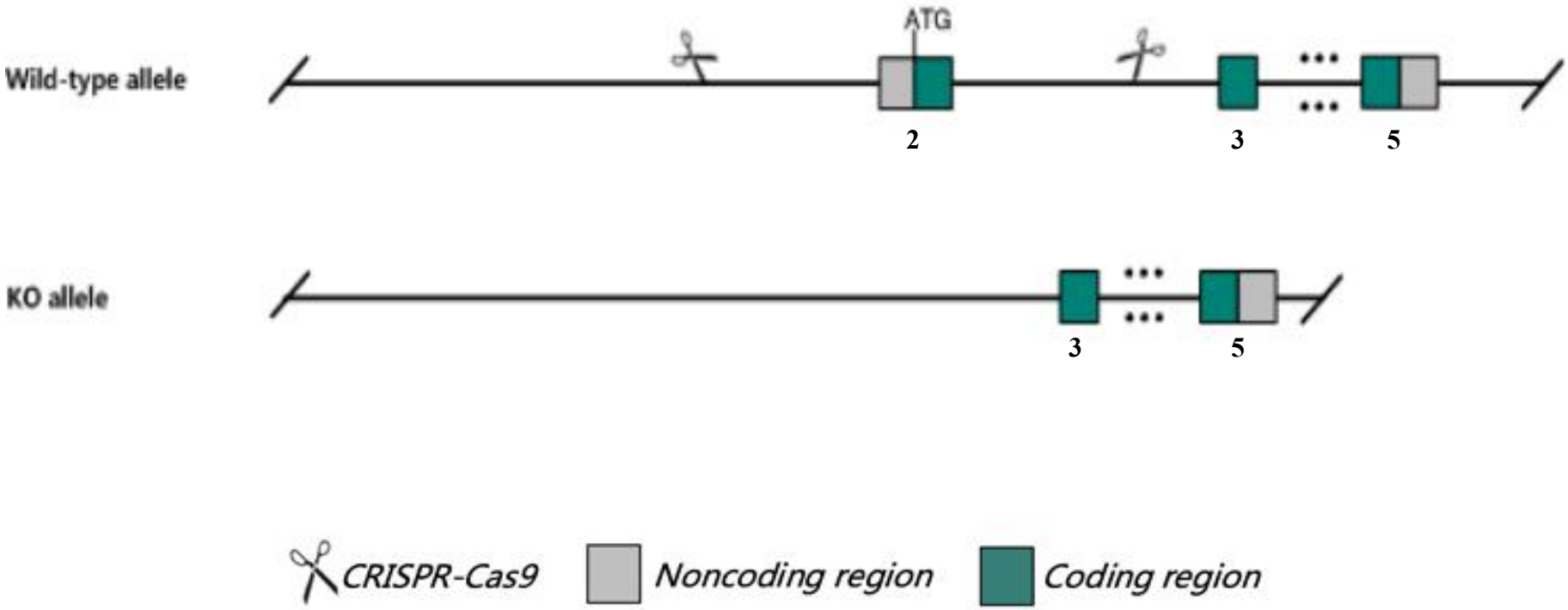
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Technical Information

- The *Zfp365* gene has 4 transcripts. According to the structure of *Zfp365* gene, exon2 of *Zfp365*-201 (ENSMUST00000064656.8) 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: gRNAs were transcribed in vitro. Cas9 and gRNAs 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.

Gene Information

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

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

Summary

Official Symbol	Zfp365 provided by MGI
Official Full Name	zinc finger protein 365 provided by MGI
Primary source	MGI:MGI:2143676
See related	Ensembl:ENSMUSG00000037855
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	AI839779, AV340892, DBZ, Su48, Znf365, talanin
Expression	Biased expression in cortex adult (RPKM 80.2), frontal lobe adult (RPKM 65.7) and 1 other tissue See more
Orthologs	human all

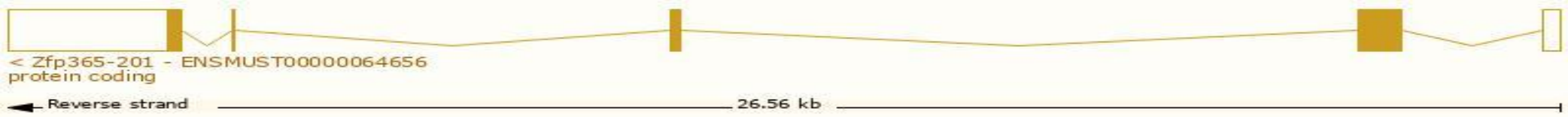
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

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

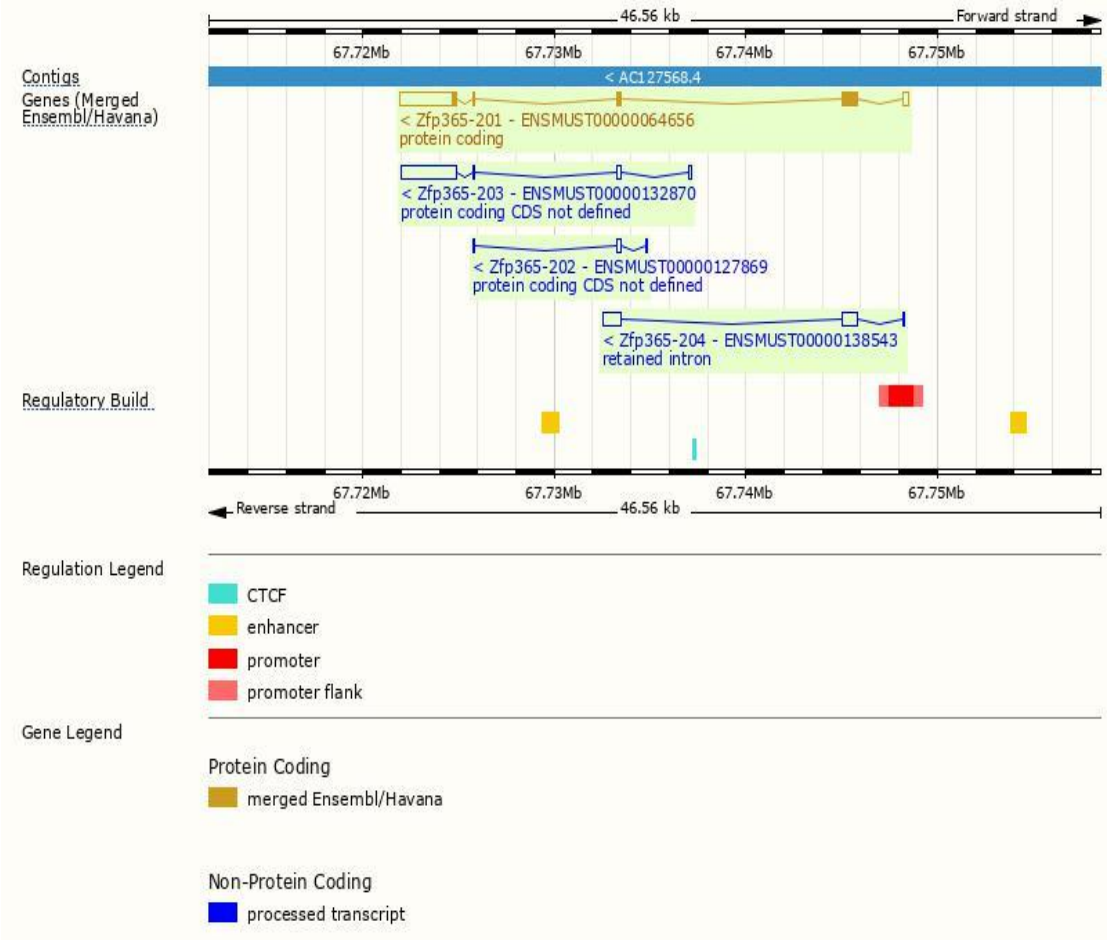
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zfp365-201	ENSMUST00000064656.7	4259	408aa	Protein coding	CCDS23903	Q8BG89	TSL:1 GENCODE basic APPRIS P1
Zfp365-204	ENSMUST00000138543.1	1745	No protein	Retained intron	-	-	TSL:1
Zfp365-203	ENSMUST00000132870.7	3276	No protein	lncRNA	-	-	TSL:1
Zfp365-202	ENSMUST00000127869.1	292	No protein	lncRNA	-	-	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



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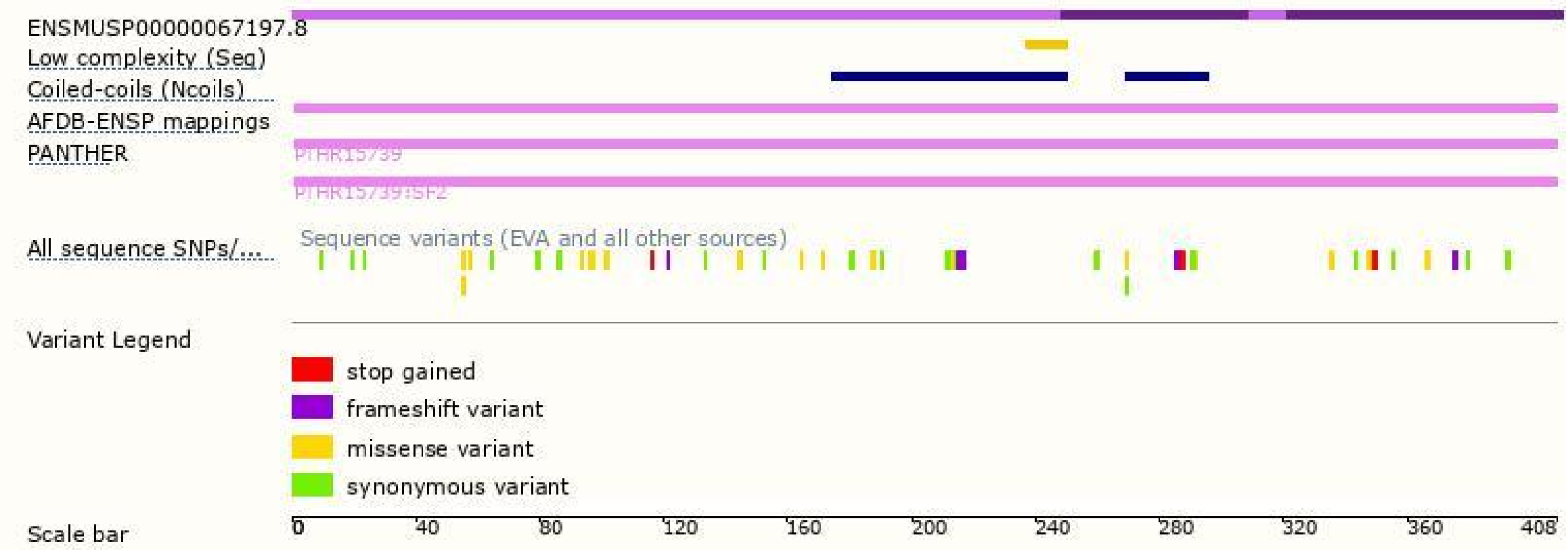
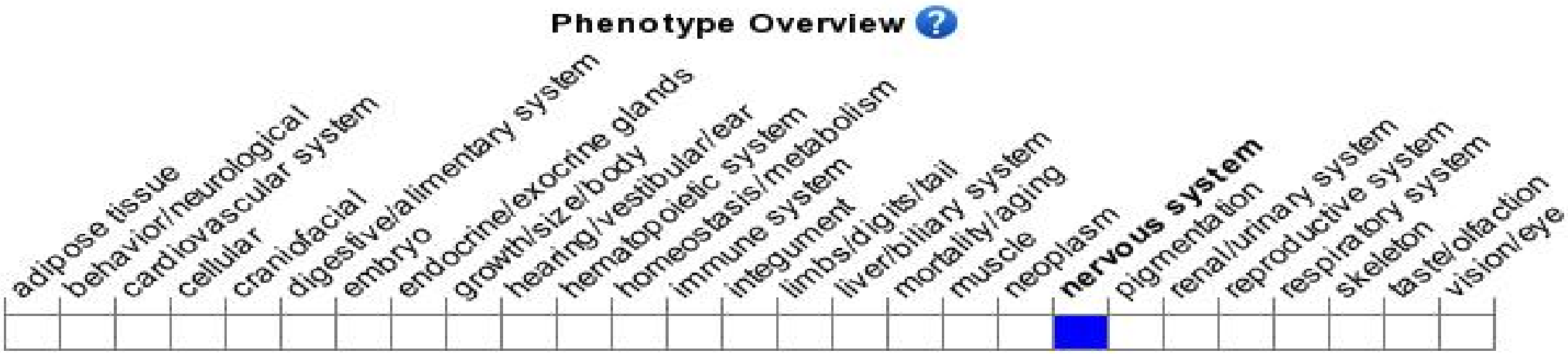
Source: : https://www.ensembl.org

Protein Information



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

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

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 risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Reference

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

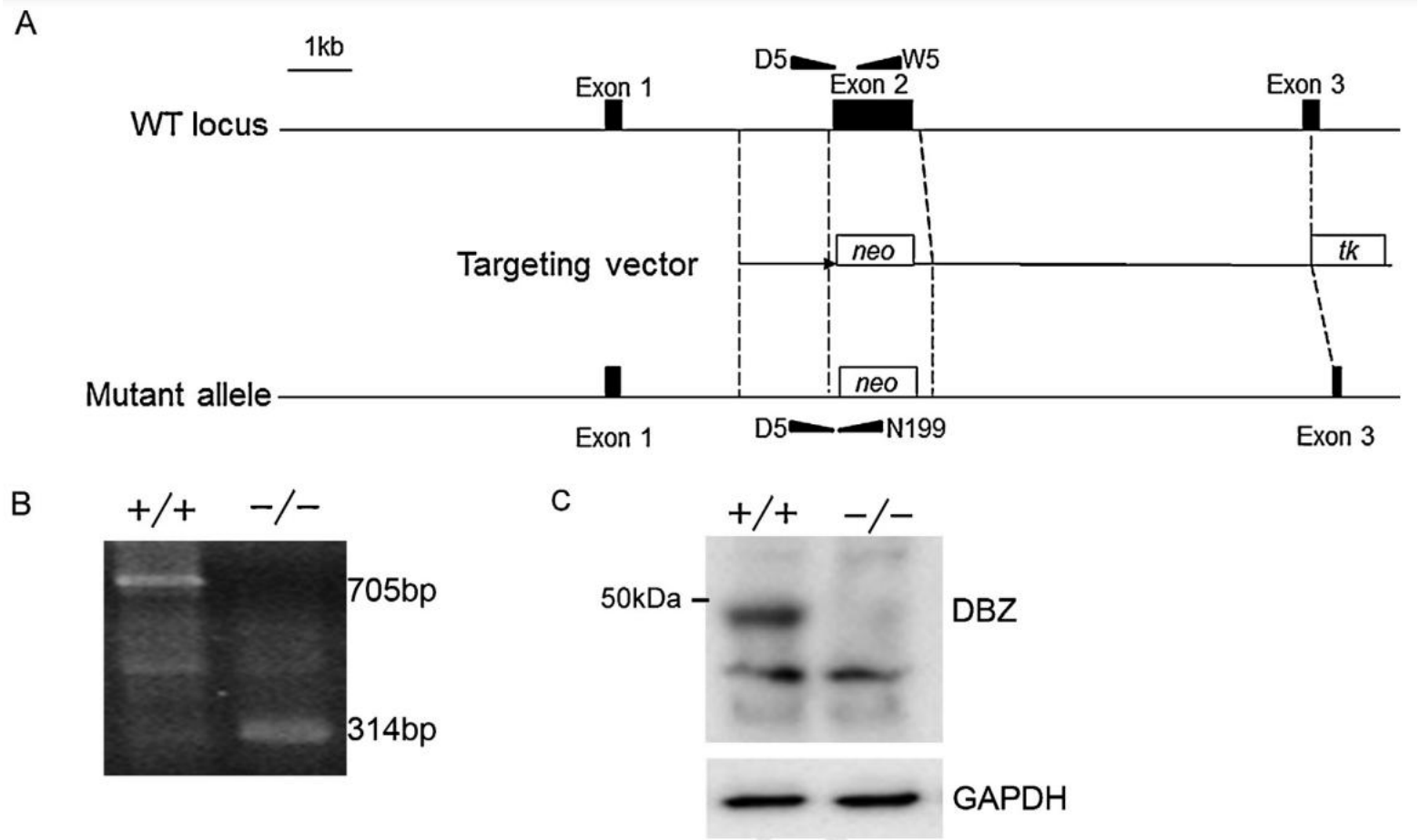


Fig. 2. Expression of DBZ in the developing brain and adult cerebral cortex. (A) *In situ* hybridization of DBZ mRNA in sagittal adult mouse brain sections at lower magnification. Scale bar, 5 mm. (B–C) *In situ* hybridization of DBZ mRNA in coronal brain sections at E14.5. DBZ mRNA is expressed both 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.