Ddhd2 Cas9-KO Strategy

Designer: Lingyan Wu

Reviewer: Miaomiao Cui

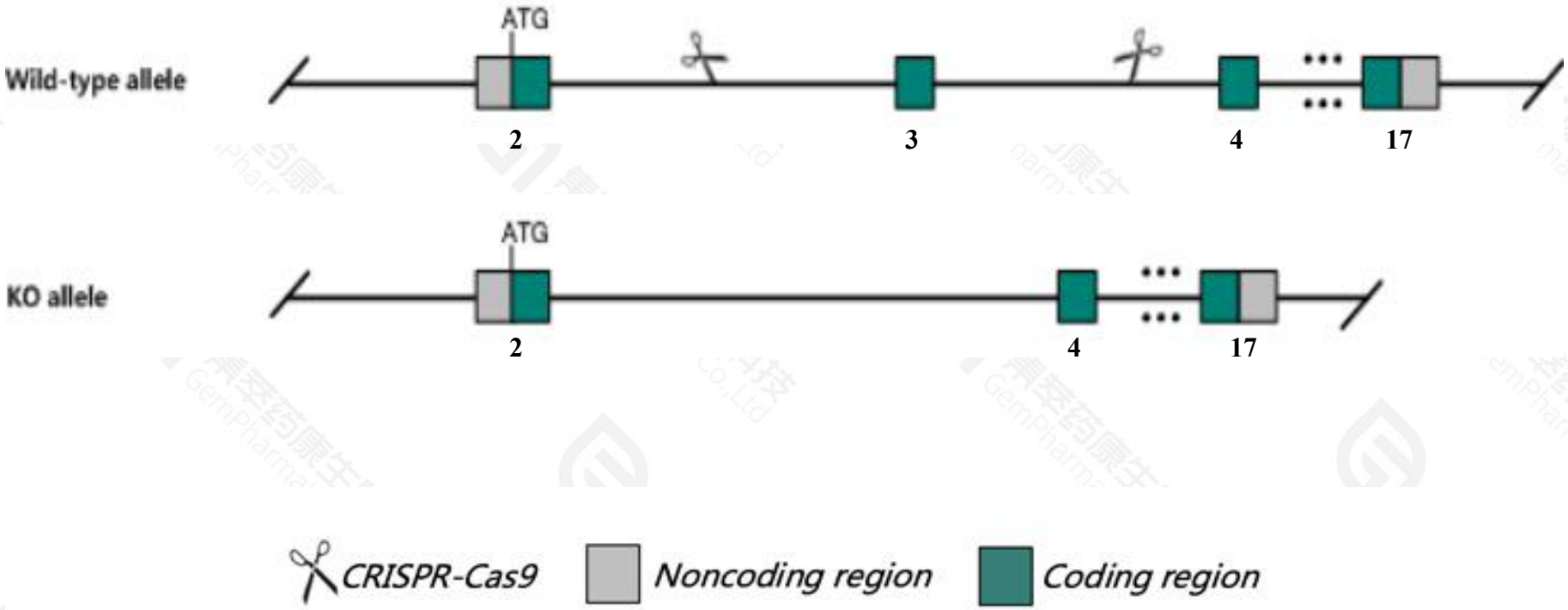
Design Date: 2022-7-11

Project Overview

Project Name	***Ddhd2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Ddhd2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ddhd2* gene has 7 transcripts. According to the structure of *Ddhd2* gene, exon3 of *Ddhd2-201*(ENSMUST00000033975.9) transcript is recommended as the knockout region.The region contains 191bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR-Cas9 technology to modify *Ddhd2* gene. The brief process is as follows: CRISPR-Cas9 system 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null mutation display impaired balance and coordination, impaired spatial learning and memory and triglyceride accumulation in neurons in the brain and spinal cord.

➢ Transcript *Ddhd2*-203&*Ddhd2*-207 may not be affected.

➢ The KO region is near to the N-terminal of *Gm17484* gene, this strategy may influence the regulatory function of the N-terminal of *Gm17484* gene.

➢ The *Ddhd2* gene is located on the Chr8. 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.

Gene information （NCBI）

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Ddhd2 DDHD domain containing 2 [Mus musculus (house mouse)]

Gene ID: 72108, updated on 13-Mar-2020

Summary

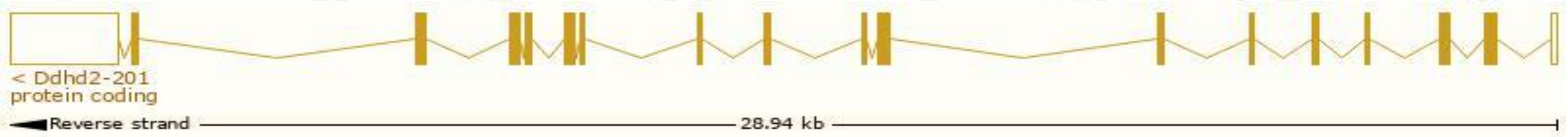
Official Symbol	Ddhd2 provided by MGI
Official Full Name	DDHD domain containing 2 provided by MGI
Primary source	MGI:MGI:1919358
See related	Ensembl:ENSMUSG00000061313
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2010305K11Rik, SAMWD1, mKIAA0725
Expression	Ubiquitous expression in cerebellum adult (RPKM 6.9), subcutaneous fat pad adult (RPKM 6.9) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

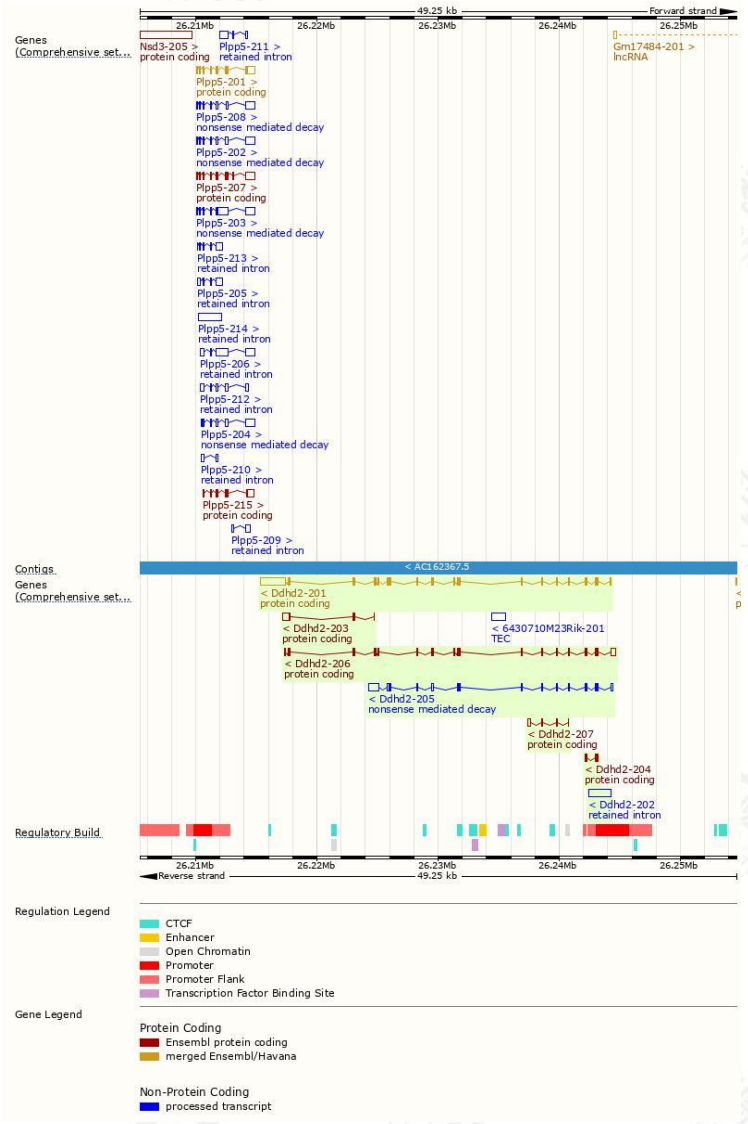
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ddhd2-201	ENSMUST00000033975.7	4245	699aa	Protein coding	CCDS52529	Q80Y98	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Ddhd2-206	ENSMUST00000211688.1	2265	730aa	Protein coding	-	A0A1B0GSA5	TSL:1 GENCODE basic
Ddhd2-203	ENSMUST00000210777.1	734	84aa	Protein coding	-	A0A1B0GT91	CDS 5' incomplete TSL:5
Ddhd2-207	ENSMUST00000211751.1	450	83aa	Protein coding	-	A0A1B0GRX3	CDS 5' incomplete TSL:2
Ddhd2-204	ENSMUST00000210888.1	339	113aa	Protein coding	-	A0A1B0GSV3	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Ddhd2-205	ENSMUST00000211009.1	2508	358aa	Nonsense mediated decay	-	A0A1B0GS27	TSL:1
Ddhd2-202	ENSMUST00000209419.1	1835	No protein	Retained intron	-	-	TSL:NA

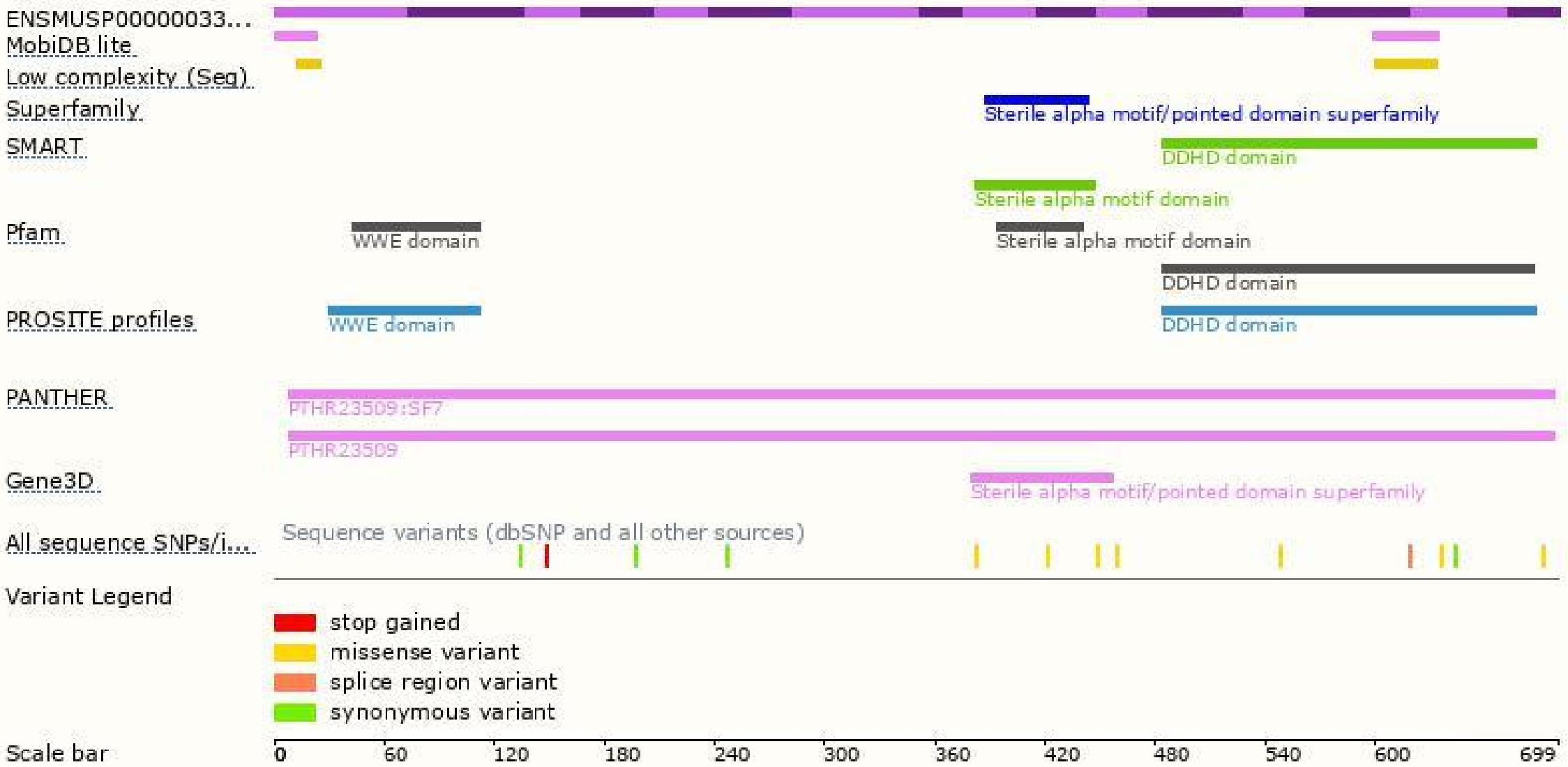
The strategy is based on the design of *Ddhd2-201* transcript,the transcription is shown below:



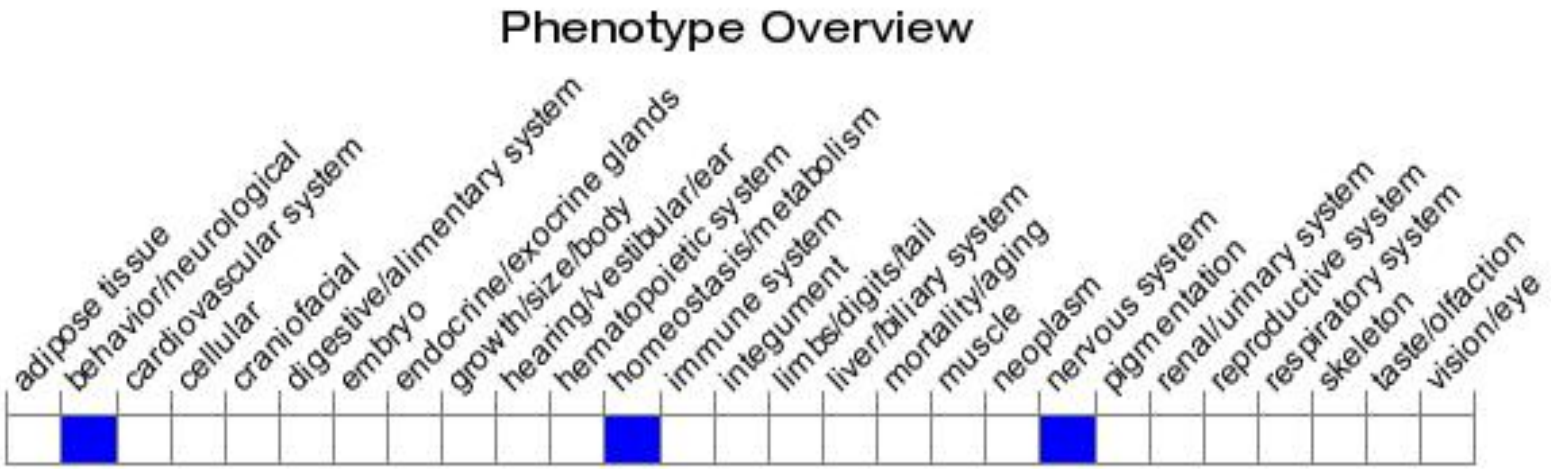
Genomic location distribution



Protein domain



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 a null mutation display impaired balance and coordination, impaired spatial learning and memory and triglyceride accumulation in neurons in the brain and spinal cord.

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

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