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Ddhd2 Cas9-CKO Strategy

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Design Date: 2023-12-7

Overview

Target Gene Name

- Ddhd2

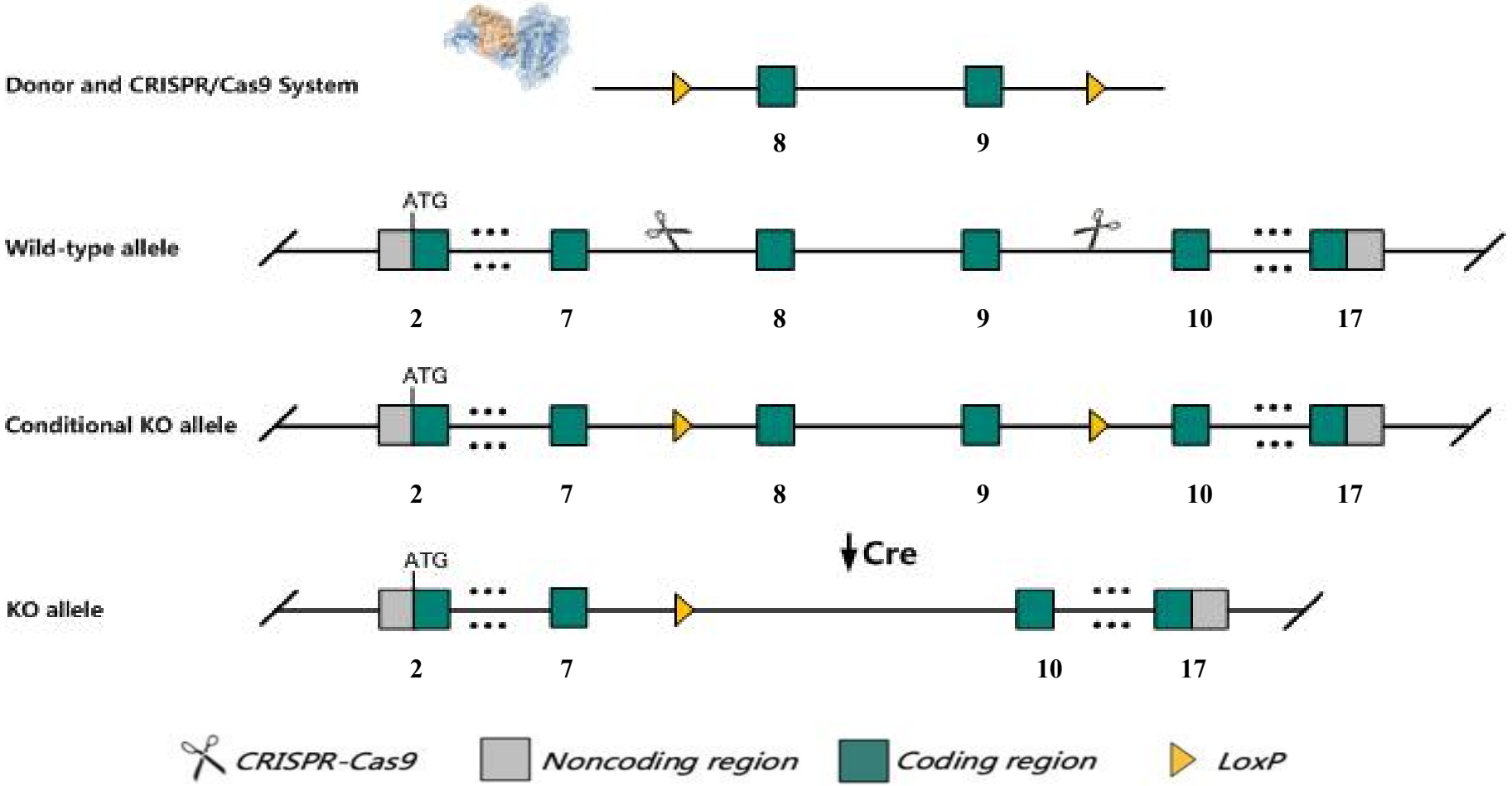
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Ddhd2* gene.

Technical Information

- The *Ddhd2* gene has 7 transcripts. According to the structure of *Ddhd2* gene, exon8-exon9 of *Ddhd2*-201 (ENSMUST00000033975.9) transcript is recommended as the knockout region. The region contains 277bp coding sequence. Knocking 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 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.

Gene Information

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

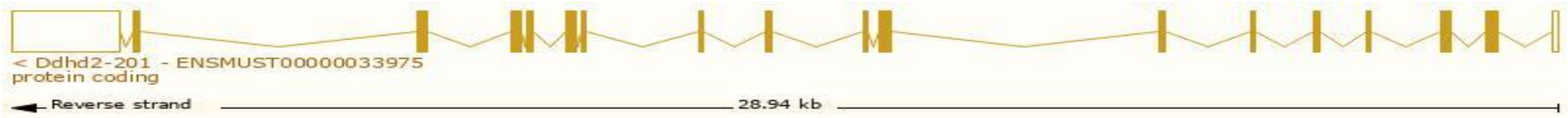
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

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

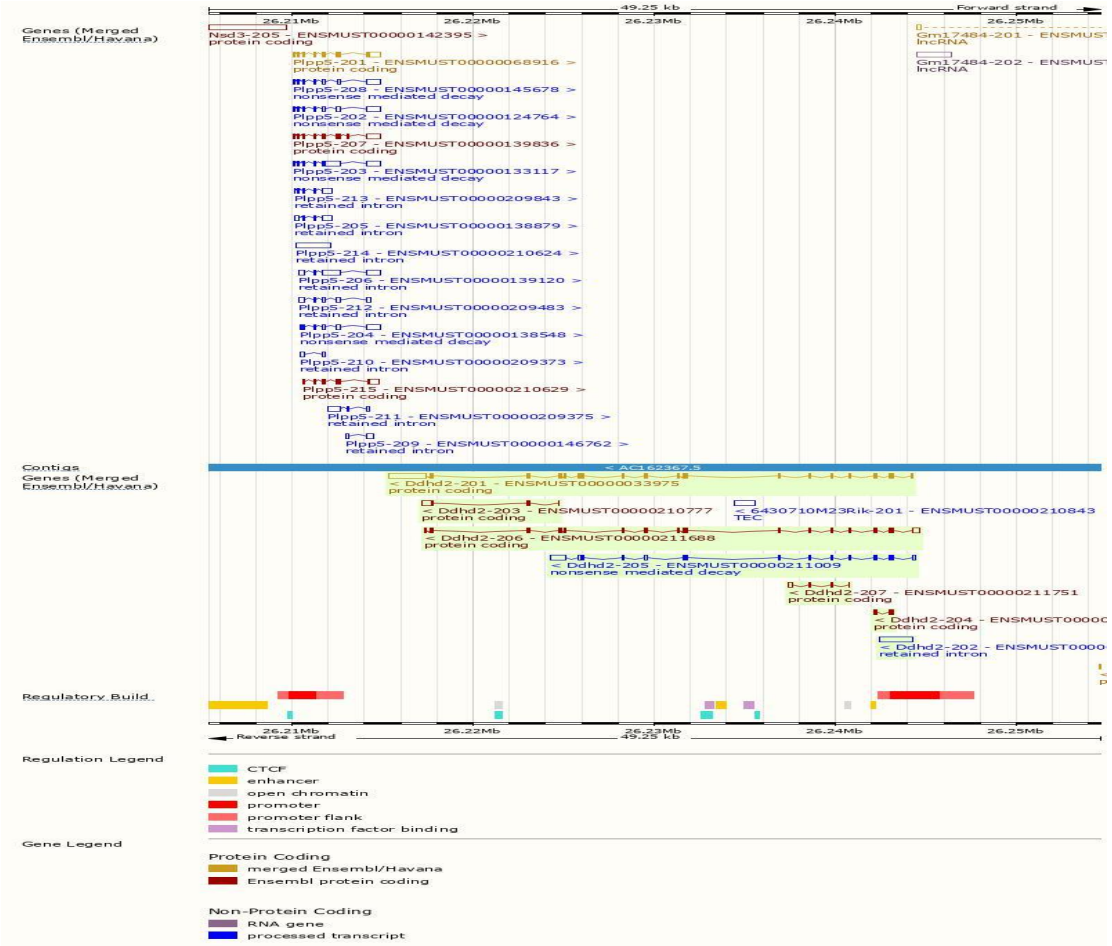
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ddhd2-201	ENSMUST00000033975.7	4245	699aa	Protein coding	CCDS52529	Q80Y98	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important 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:



Source: https://www.ensembl.org

Genomic Information



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

Protein Information

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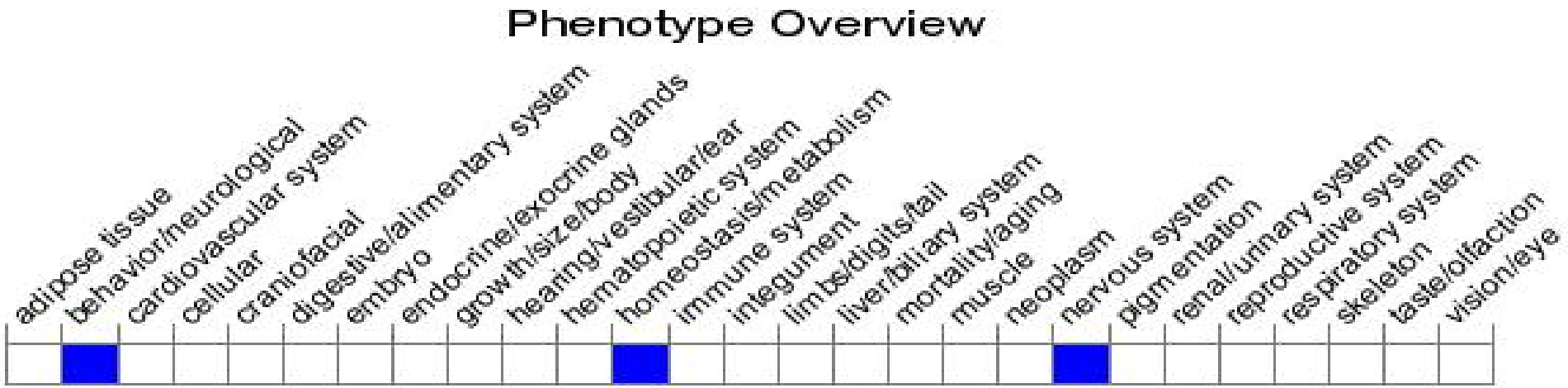
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



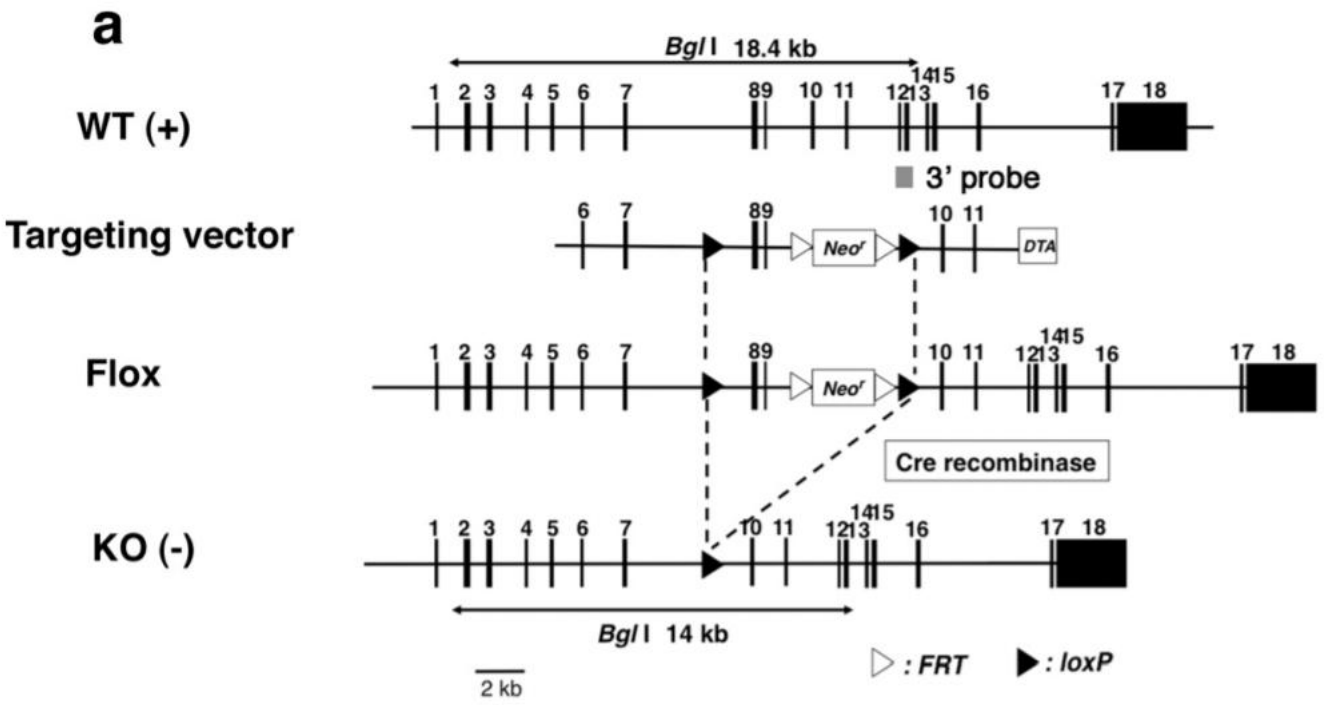
- Mice homozygous for a null mutation display impaired balance and coordination, impaired spatial learning and memory and triglyceride accumulation in neurons in the brain and spinal cord.

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

Important Information

- According to MGI information, 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.
- The N-terminal of *Ddhd2* gene will remain several amino acids, it may remain the partial function of *Ddhd2* gene.
- Transcript *Ddhd2*-203, *Ddhd2*-204, *Ddhd2*-207 may not be affected.
- *Ddhd2* is located on Chr8. 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



Results

Loss of motor neurons in the spinal cords of *DDHD2* KO mice

To reveal the physiological function of DDHD2, we generated *DDHD2* KO mice. Using a targeting vector that contains exons 8 and 9 flanked by two *loxP* sites (Supplementary Figure 1a), we obtained targeted cell lines, and then generated chimeric, flox, and heterozygous and homozygous KO mice, as described under Materials and methods. Southern and Western blotting (WB)

Maruyama T, Baba T, Maemoto Y, Hara-Miyauchi C, Hasegawa-Ogawa M, Okano HJ, Enda Y, Matsumoto K, Arimitsu N, Nakao K, Hamamoto H, Sekimizu K, Ohto-Nakanishi T, Nakanishi H, Tokuyama T, Yanagi S, Tagaya M, Tani K, Loss of DDHD2, whose mutation causes spastic paraplegia, promotes reactive oxygen species generation and apoptosis. Cell Death Dis. 2018 Jul 23;9(8):797