

Wdfy3 Cas9-CKO Strategy

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Design Date:2020-2-27

Project Overview



Project Name

Wdfy3

Project type

Cas9-CKO

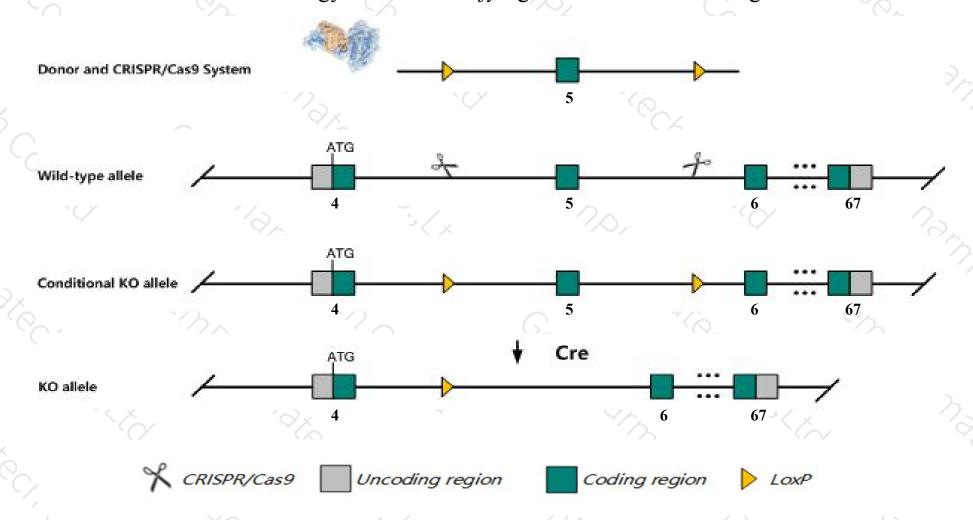
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Wdfy3 gene. The schematic diagram is as follows:



Technical routes



- The *Wdfy3* gene has 7 transcripts. According to the structure of *Wdfy3* gene, exon5 of *Wdfy3-201*(ENSMUST00000053177.13) transcript is recommended as the knockout region. The region contains 124bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Wdfy3* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- 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.

Notice



- ➤ According to the existing MGI data, Mice homozygous for hypomorphic mutations of this gene exhibit perinatal lethality, altered neural progenitor divisions and neuronal migration, a regionally enlarged cerebral cortex, and focal cortical dysplasias.
- > The *Wdfy3* gene is located on the Chr5. 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)



Wdfy3 WD repeat and FYVE domain containing 3 [Mus musculus (house mouse)]

Gene ID: 72145, updated on 19-Feb-2019

Summary

☆ ?

Official Symbol Wdfy3 provided by MGI

Official Full Name WD repeat and FYVE domain containing 3 provided by MGI

Primary source MGI:MGI:1096875

See related Ensembl:ENSMUSG00000043940

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 2610509D04Rik, ALFY, AW319683, B930017C24, BWF1, Bchs, D5Ertd66e, Ggtb3, ZFYVE25, mKIAA0993

Expression Ubiquitous expression in cerebellum adult (RPKM 13.9), whole brain E14.5 (RPKM 11.6) and 28 other tissuesSee more

Orthologs <u>human</u> all

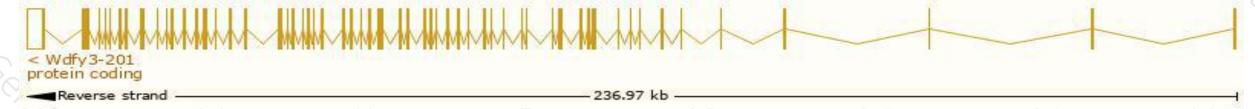
Transcript information (Ensembl)



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

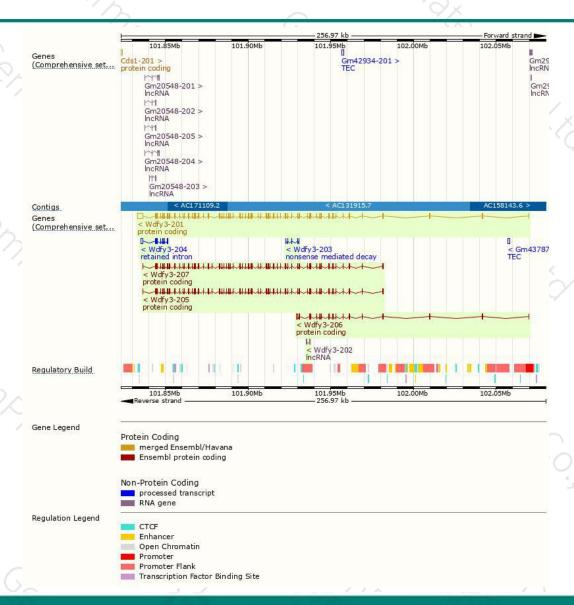
offin.						
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000053177.13	14274	3508aa	Protein coding	CCDS19473	Q6VNB8	TSL:1 GENCODE basic APPRIS P2
ENSMUST00000174598.7	10581	3526aa	Protein coding	37.5	G3UYW1	TSL:5 GENCODE basic APPRIS ALT1
ENSMUST00000212024.1	10539	<u>3512aa</u>	Protein coding	829	A0A1D5RLV7	TSL:5 GENCODE basic
ENSMUST00000174698.1	3858	<u>913aa</u>	Protein coding	3528	Q6VNB8	TSL:1 GENCODE basic
ENSMUST00000172927.1	641	<u>124aa</u>	Nonsense mediated decay	187	G3UY81	CDS 5' incomplete TSL:5
ENSMUST00000173955.1	2713	No protein	Retained intron	37.5	-	TSL:1
ENSMUST00000172512.1	704	No protein	IncRNA	(2)	2	TSL:3
	ENSMUST00000053177.13 ENSMUST00000174598.7 ENSMUST00000212024.1 ENSMUST00000174698.1 ENSMUST00000172927.1 ENSMUST00000173955.1	ENSMUST00000053177.13 14274 ENSMUST00000174598.7 10581 ENSMUST00000212024.1 10539 ENSMUST00000174698.1 3858 ENSMUST00000172927.1 641 ENSMUST00000173955.1 2713	ENSMUST00000053177.13 14274 3508aa ENSMUST00000174598.7 10581 3526aa ENSMUST00000212024.1 10539 3512aa ENSMUST00000174698.1 3858 913aa ENSMUST00000172927.1 641 124aa ENSMUST00000173955.1 2713 No protein	ENSMUST00000053177.13 14274 3508aa Protein coding ENSMUST00000174598.7 10581 3526aa Protein coding ENSMUST00000212024.1 10539 3512aa Protein coding ENSMUST00000174698.1 3858 913aa Protein coding ENSMUST000000172927.1 641 124aa Nonsense mediated decay ENSMUST000000173955.1 2713 No protein Retained intron	ENSMUST00000053177.13 14274 3508aa Protein coding CCDS19473 ENSMUST00000174598.7 10581 3526aa Protein coding - ENSMUST00000212024.1 10539 3512aa Protein coding - ENSMUST00000174698.1 3858 913aa Protein coding - ENSMUST00000172927.1 641 124aa Nonsense mediated decay - ENSMUST00000173955.1 2713 No protein Retained intron -	ENSMUST00000053177.13 14274 3508aa Protein coding CCDS19473 Q6VNB8 ENSMUST00000174598.7 10581 3526aa Protein coding - G3UYW1 ENSMUST00000212024.1 10539 3512aa Protein coding - A0A1D5RLV7 ENSMUST00000174698.1 3858 913aa Protein coding - Q6VNB8 ENSMUST00000172927.1 641 124aa Nonsense mediated decay - G3UY81 ENSMUST00000173955.1 2713 No protein Retained intron - - -

The strategy is based on the design of *Wdfy3-201* transcript, The transcription is shown below



Genomic location distribution





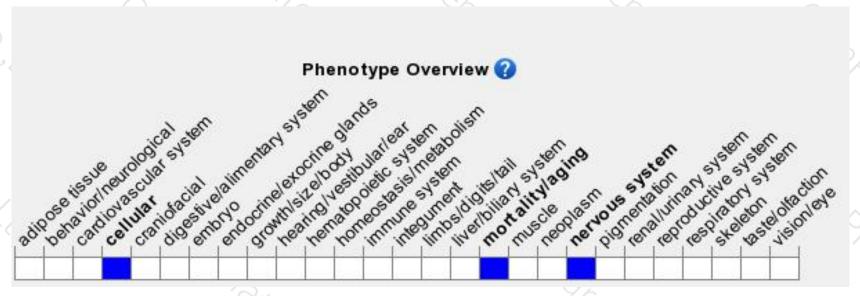
Protein domain





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 hypomorphic mutations of this gene exhibit perinatal lethality, altered neural progenitor divisions and neuronal migration, a regionally enlarged cerebral cortex, and focal cortical dysplasias.



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





