

Mef2d Cas9-CKO Strategy

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Reviewer: Huimin Su

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Project Overview



Project Name Mef2d

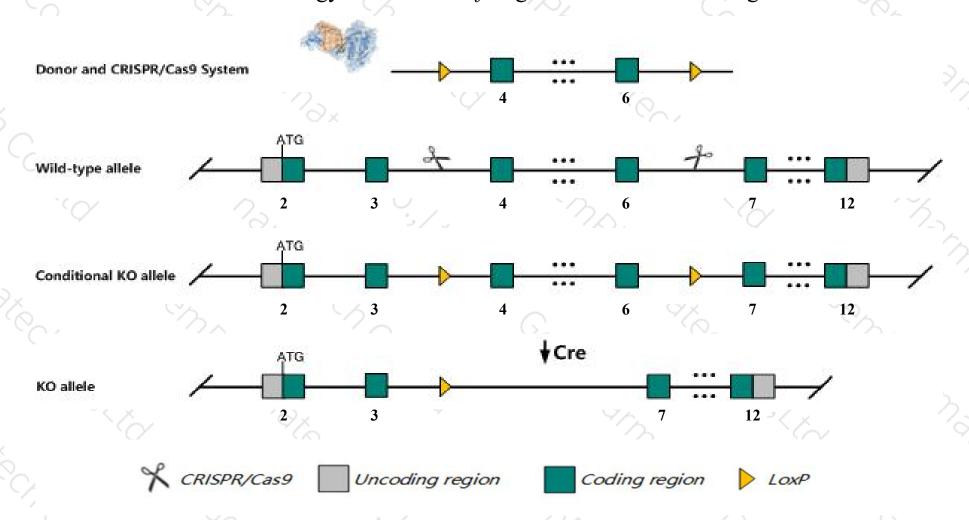
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Mef2d* gene. The schematic diagram is as follows:



Technical routes



- The *Mef2d* gene has 7 transcripts. According to the structure of *Mef2d* gene, exon4-exon6 of *Mef2d-203* (ENSMUST00000107559.2) transcript is recommended as the knockout region. The region contains 406bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Mef2d* 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 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 a knock-out allele exhibit abnormal synapse formation between retinal photoreceptor and bipolar cells, progressive photoreceptor degeneration, and severely impaired electroretinograms.
- > The *Mef2d* gene is located on the Chr3. 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)



Mef2d myocyte enhancer factor 2D [Mus musculus (house mouse)]

Gene ID: 17261, updated on 15-Mar-2020

Summary

2

Official Symbol Mef2d provided by MGI

Official Full Name myocyte enhancer factor 2D provided by MGI

Primary source MGI:MGI:99533

See related Ensembl: ENSMUSG00000001419

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 C80750

Expression Ubiquitous expression in thymus adult (RPKM 72.5), ovary adult (RPKM 42.1) and 28 other tissues See more

Orthologs human all

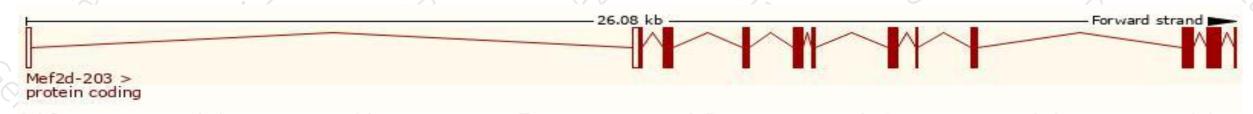
Transcript information (Ensembl)



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

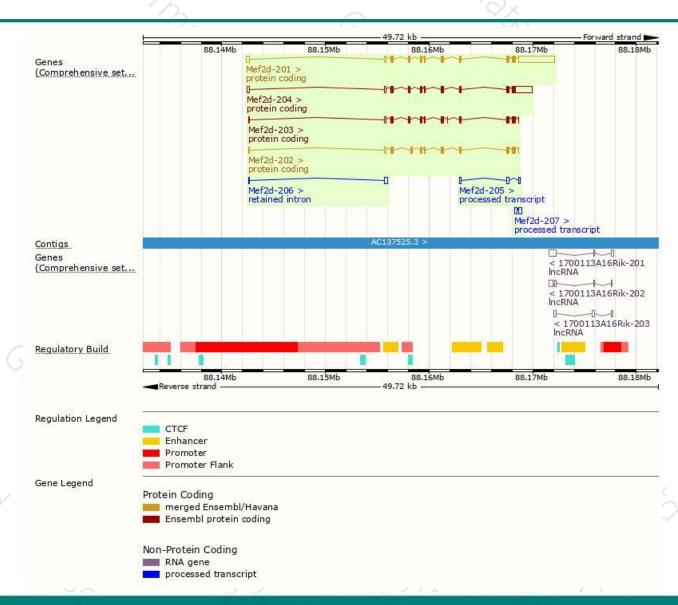
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mef2d-201	ENSMUST00000001455.12	5401	507aa	Protein coding	CCDS38479	Q921S6	TSL:1 GENCODE basic
Mef2d-203	ENSMUST00000107559.2	1791	<u>514aa</u>	Protein coding	CCDS79939	Q63943	TSL:1 GENCODE basic
Mef2d-202	ENSMUST00000107558.8	1767	506aa	Protein coding	CCDS79940	E9QKT0	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 P
Mef2d-204	ENSMUST00000119251.7	3488	506aa	Protein coding	-	E9Q5E0	TSL:5 GENCODE basic
Mef2d-205	ENSMUST00000139153.1	502	No protein	Processed transcript	-	-	TSL:2
Mef2d-207	ENSMUST00000152861.1	482	No protein	Processed transcript		-	TSL:2
Mef2d-206	ENSMUST00000140927.1	427	No protein	Retained intron	(4)	-	TSL:2

The strategy is based on the design of *Mef2d-203* transcript, the transcription is shown below:



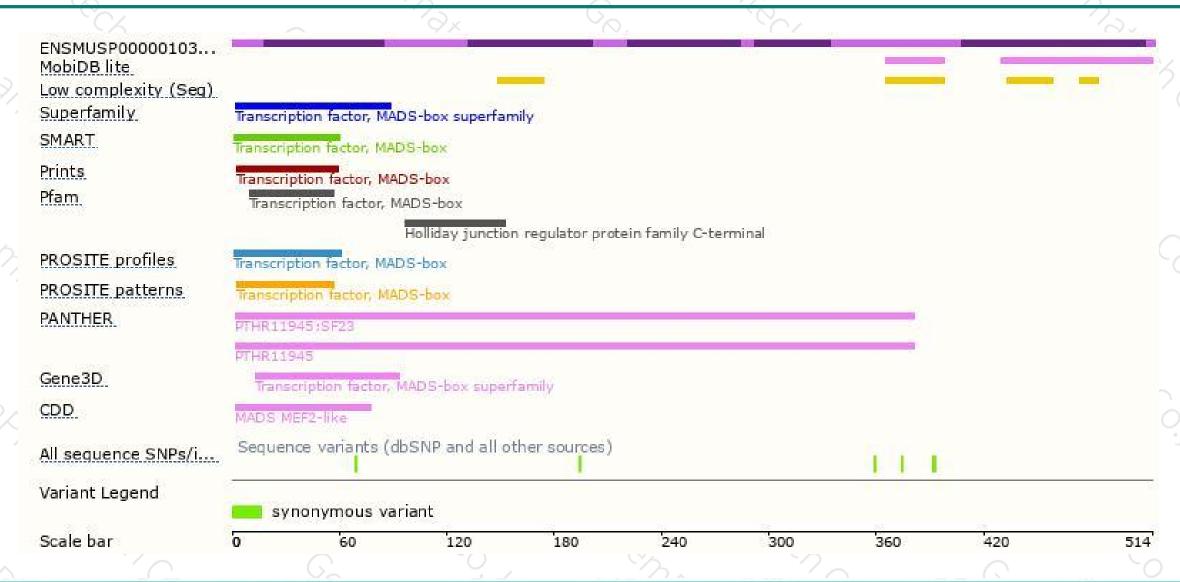
Genomic location distribution





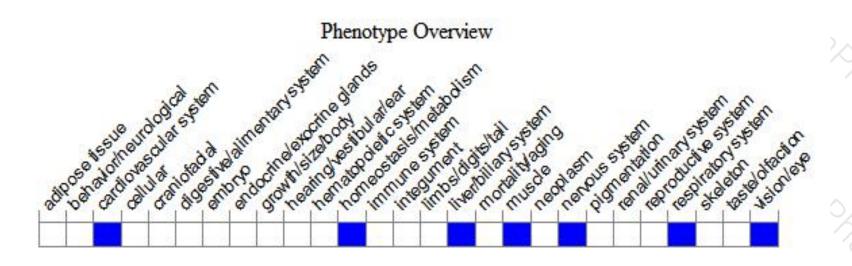
Protein domain





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 knock-out allele exhibit abnormal synapse formation between retinal photoreceptor and bipolar cells, progressive photoreceptor degeneration, and severely impaired electroretinograms.



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





