

***Fat3* Cas9-CKO Strategy**

Designer:

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Project Overview

Project Name

Fat3

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

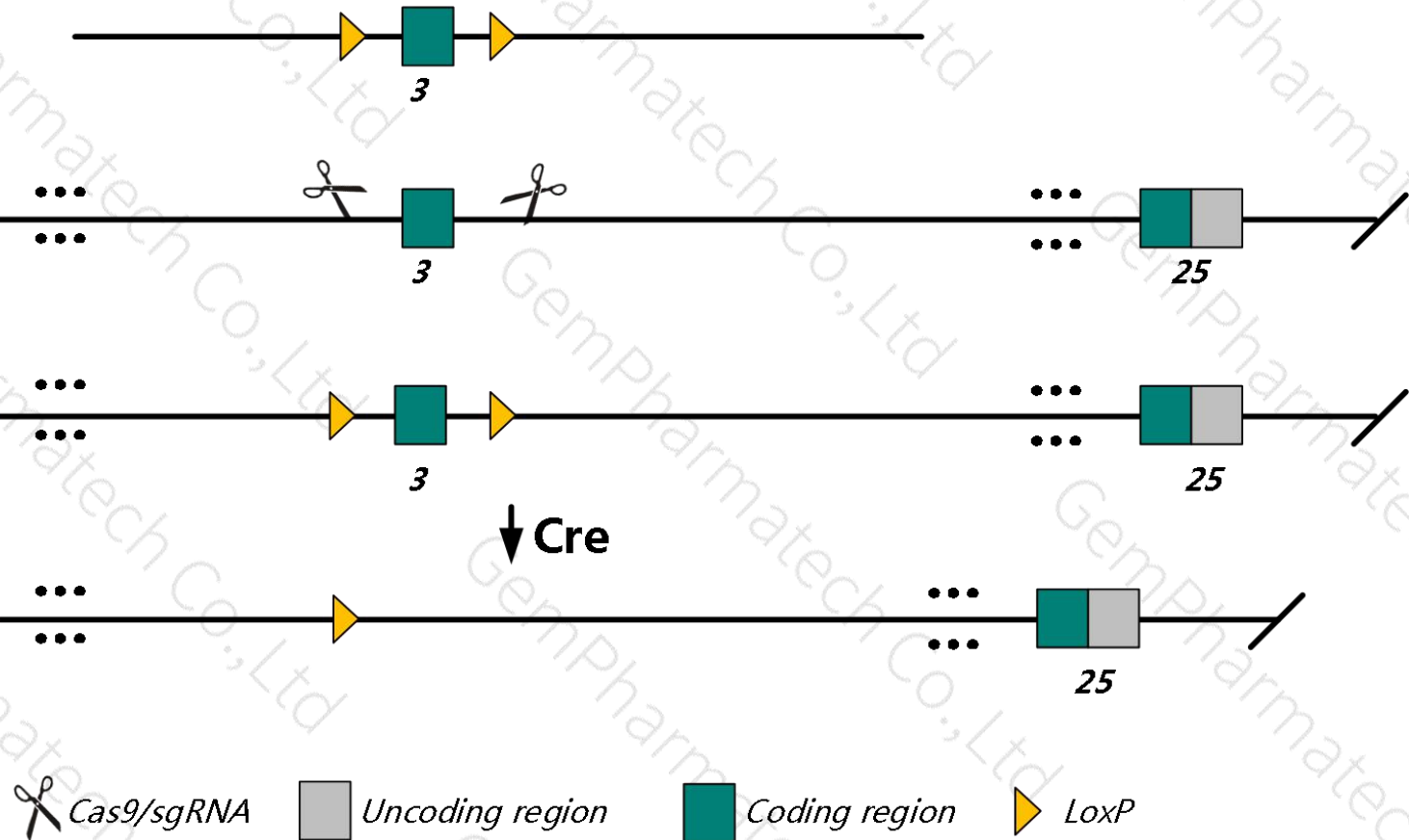
This model will use CRISPR/Cas9 technology to edit the *Fat3* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



Technical routes

- The *Fat3* gene has 5 transcripts. According to the structure of *Fat3* gene, exon4 of *Fat3-201* (ENSMUST00000082170.5) transcript is recommended as the knockout region. The region contains 62bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Fat3* 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.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit abnormal amacrine cell differentiation and migration that result in the formation of two additional plexiform layers and thickened retinal ganglion layer.
- The *Fat3* gene is located on the Chr9. 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)

Fat3 FAT atypical cadherin 3 [Mus musculus (house mouse)]

Gene ID: 270120, updated on 12-Mar-2019

Summary



Official Symbol	Fat3 provided by MGI
Official Full Name	FAT atypical cadherin 3 provided by MGI
Primary source	MGI:MGI:2444314
See related	Ensembl:ENSMUSG00000074505
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	9430076A06Rik, D430038H04Rik, Gm1132, Gm510, MFAT3F
Expression	Biased expression in whole brain E14.5 (RPKM 3.7), frontal lobe adult (RPKM 3.2) and 12 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

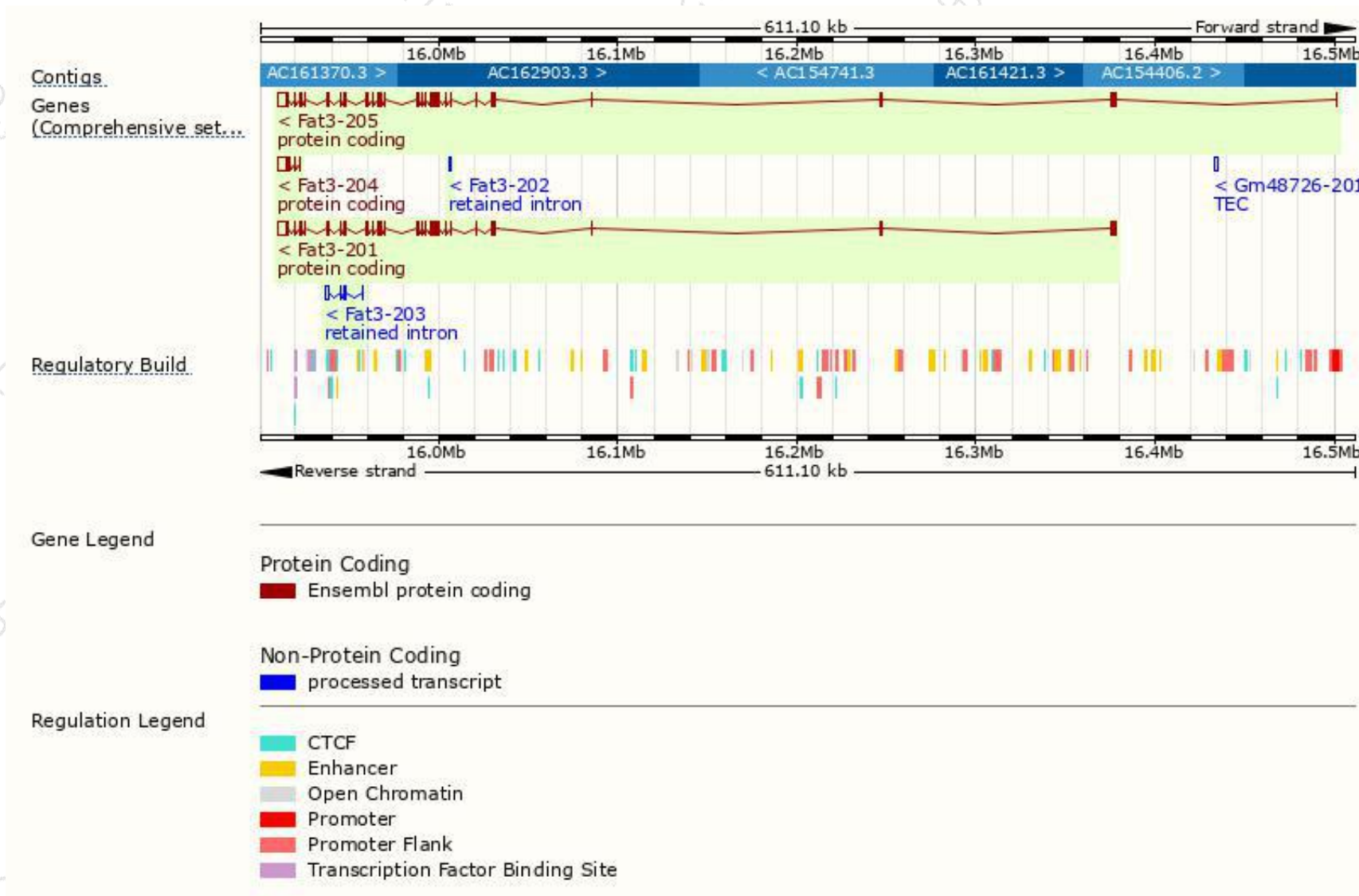
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fat3-205	ENSMUST00000217308.1	18760	4551aa	Protein coding	CCDS40539	E9QK16	TSL:5 GENCODE basic APPRIS P1
Fat3-201	ENSMUST00000082170.5	18456	4551aa	Protein coding	CCDS40539	E9QK16	TSL:5 GENCODE basic APPRIS P1
Fat3-204	ENSMUST00000217187.1	6024	404aa	Protein coding	-	A0A1L1SU86	CDS 5' incomplete TSL:3
Fat3-203	ENSMUST00000215388.1	2214	No protein	Retained intron	-	-	TSL:1
Fat3-202	ENSMUST00000213517.1	1391	No protein	Retained intron	-	-	TSL:NA

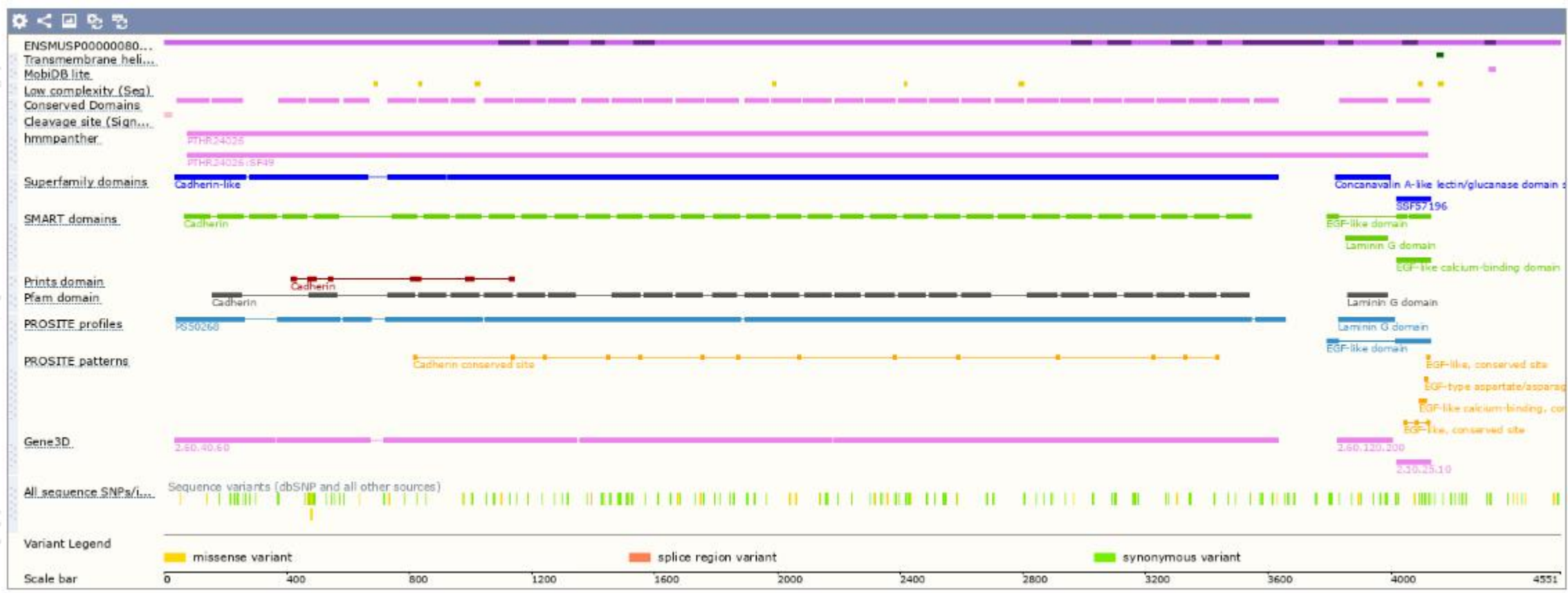
The strategy is based on the design of *Fat3-201* transcript,The transcription is shown below



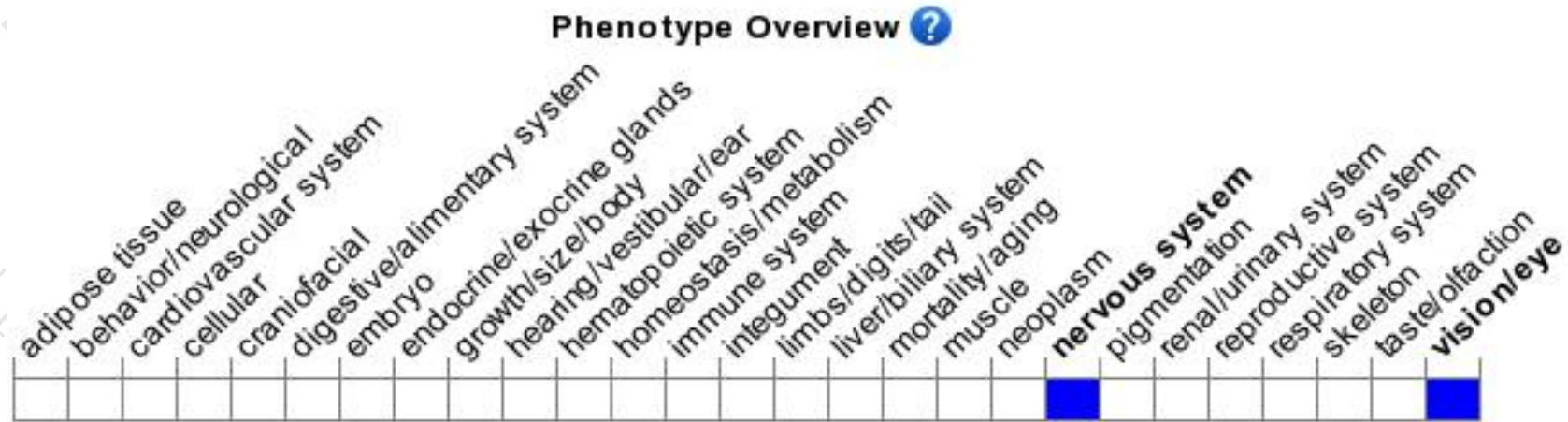
Genomic location distribution



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 knock-out allele exhibit abnormal amacrine cell differentiation and migration that result in the formation of two additional plexiform layers and thickened retinal ganglion layer.

If you have any questions, you are welcome to inquire.

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