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Fa2h Cas9-CKO Strategy

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Reviewer: Yanhua Shen

Design Date: 2023-2-17

Overview

Target Gene Name

- Fa2h

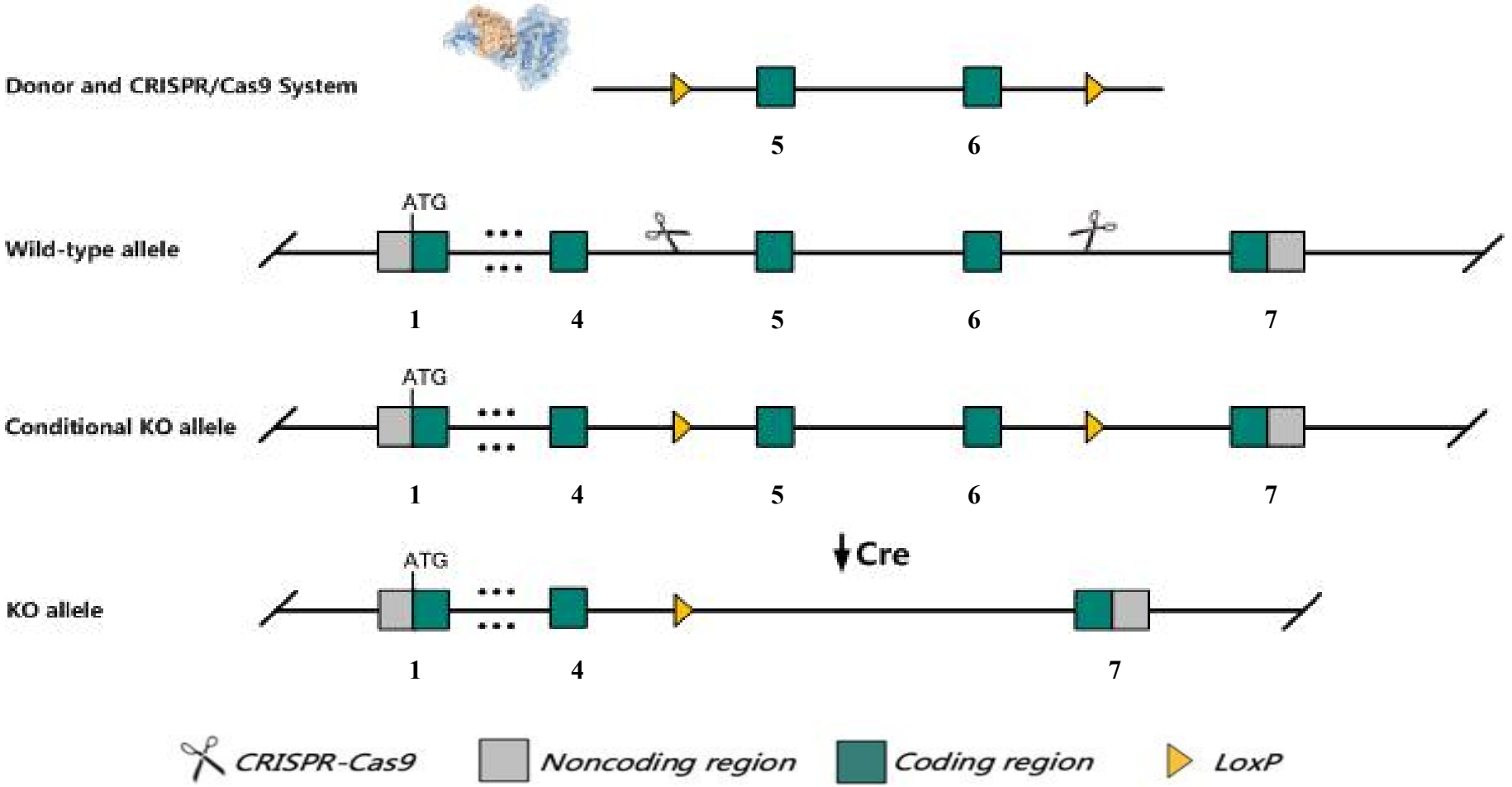
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Fa2h* gene.

Technical Information

- The *Fa2h* gene has 4 transcripts. According to the structure of *Fa2h* gene, exon5-exon6 of *Fa2h-201*(ENSMUST00000038475.9) transcript is recommended as the knockout region.The region contains 426bp coding sequence. Knock out the region may result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Fa2h* 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

Fa2h fatty acid 2-hydroxylase [Mus musculus (house mouse)]

Gene ID: 338521, updated on 19-Mar-2019

Summary

Official Symbol	Fa2h provided by MGI
Official Full Name	fatty acid 2-hydroxylase provided by MGI
Primary source	MGI:MGI:2443327
See related	Ensembl:ENSMUSG00000033579
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	FAAH, Faxdc1, G630055L08Rik
Expression	Biased expression in stomach adult (RPKM 77.1), colon adult (RPKM 59.1) and 8 other tissues See more
Orthologs	human all

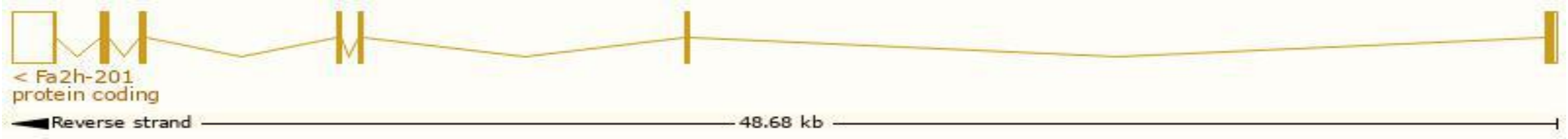
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

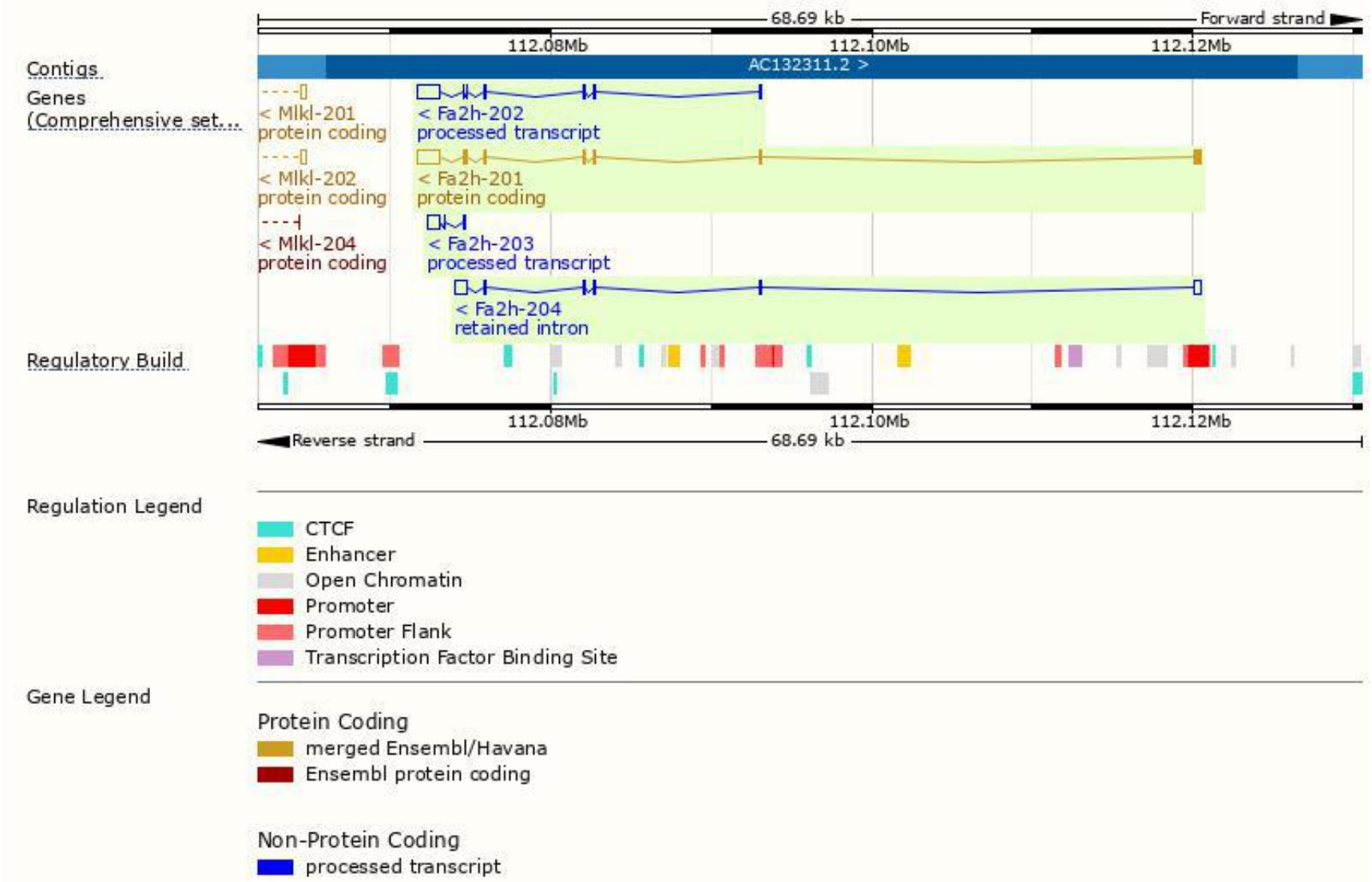
The gene has 4 transcripts, the transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fa2h-201	ENSMUST00000038475.8	2492	372aa	Protein coding	CCDS22674	Q5MPP0	TSL:1 GENCODE basic APPRIS P1
Fa2h-204	ENSMUST00000162463.1	1566	No protein	Retained intron	-	-	TSL:1
Fa2h-202	ENSMUST00000159336.7	1971	No protein	lncRNA	-	-	TSL:5
Fa2h-203	ENSMUST00000162216.1	933	No protein	lncRNA	-	-	TSL:3

The strategy is based on the design of *Fa2h*-201 transcript, the transcription is shown below:



Genomic Information



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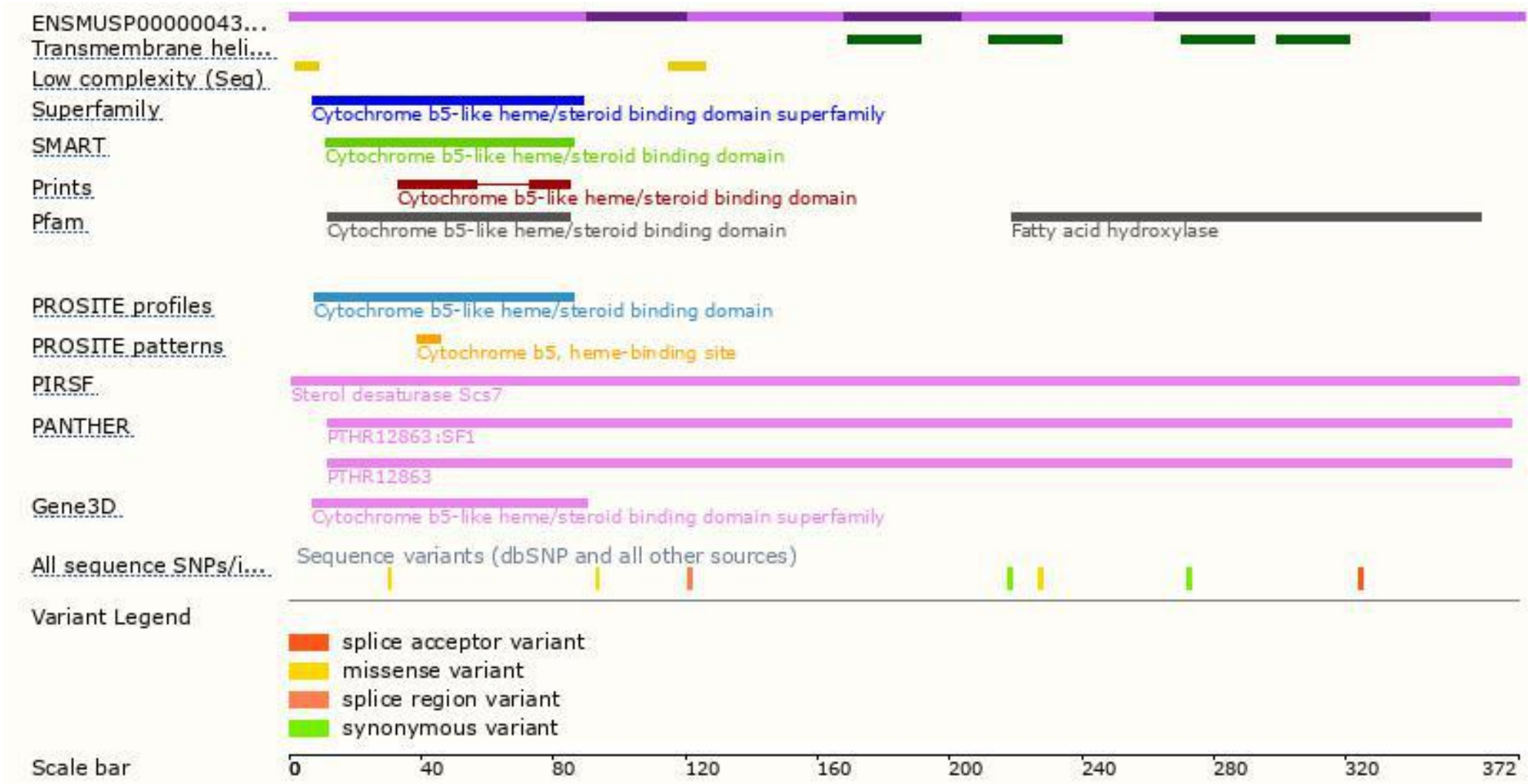
Source: : https://www.ensembl.org

Protein Information



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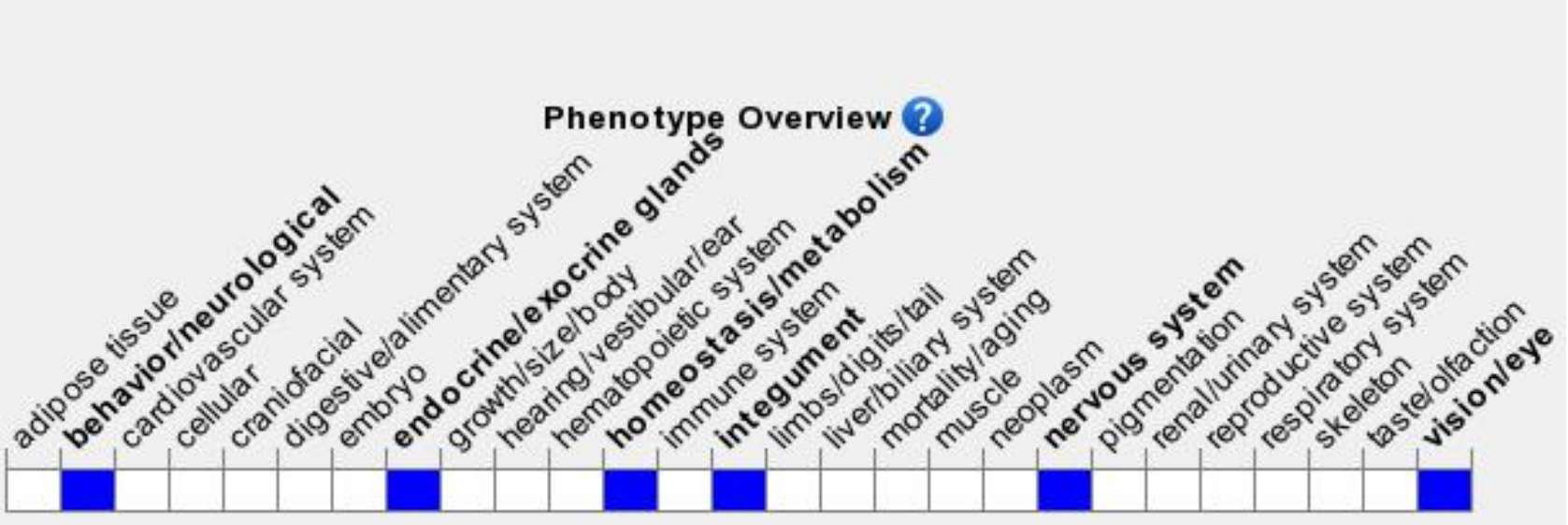
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- According to the existing MGI data,homozygotes for a null allele show demyelination, axonal loss, and cerebellar dysfunction. Homozygotes for a different null allele show late onset axon and myelin sheath degeneration, delayed fur emergence, altered sebum composition, sebocyte hyperproliferation, and cyclic alopecia.

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

Important Information

- According to the existing MGI data,homozygotes for a null allele show demyelination, axonal loss, and cerebellar dysfunction. Homozygotes for a different null allele show late onset axon and myelin sheath degeneration, delayed fur emergence, altered sebum composition, sebocyte hyperproliferation, and cyclic alopecia.
- The effect of the floxed region(426bp) without frameshift is unknown in this strategy.
- The N-terminal of *Fa2h* gene will remain some amino acids,it may remain the partial function of *Fa2h* gene.
- *Fa2h* 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.