

***Fat1* Cas9-CKO Strategy**

Designer:

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Design Date:

2019-7-17

Project Overview

Project Name

Fat1

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

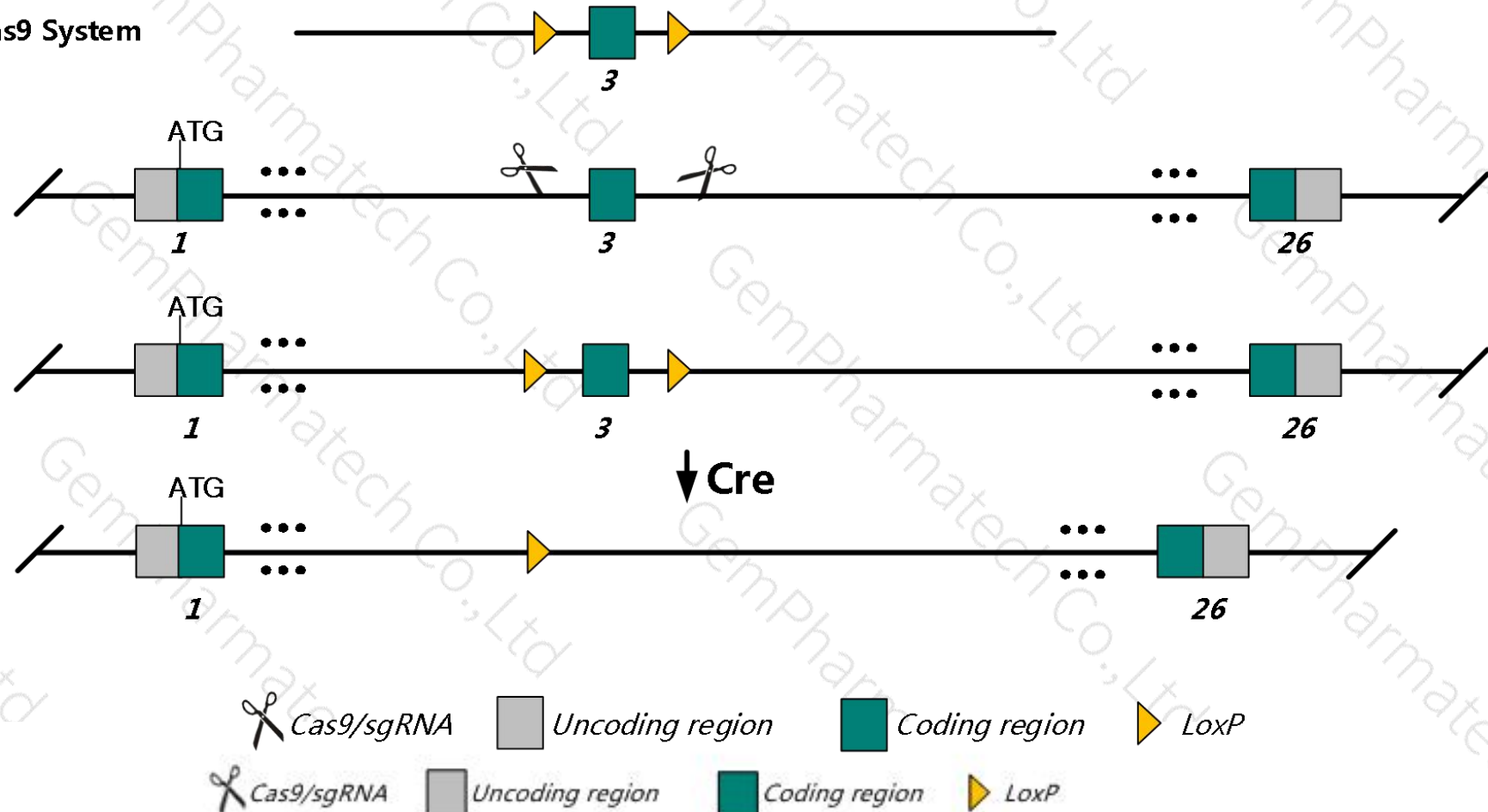
This model will use CRISPR/Cas9 technology to edit the *Fat1* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



Technical routes

- The *Fat1* gene has 6 transcripts. According to the structure of *Fat1* gene, exon3 of *Fat1-201* (ENSMUST00000098796.9) 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 *Fat1* 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, Homozygotes for a targeted null mutation exhibit holoprosencephaly, anophthalmia, kidney defects and perinatal lethality. Mice homozygous for a hypomorphic allele exhibit altered shoulder girdle and facial musculature, retinal defects, abnormal inner ear patterning and kidney defects.
- The *Fat1* gene is located on the Chr8. 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)

Fat1 FAT atypical cadherin 1 [Mus musculus (house mouse)]

Gene ID: 14107, updated on 31-Jan-2019

Summary



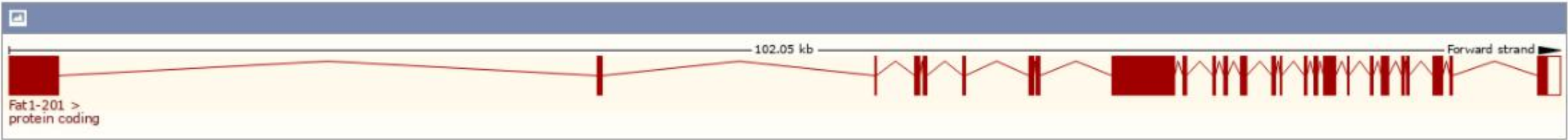
Official Symbol	Fat1 provided by MGI
Official Full Name	FAT atypical cadherin 1 provided by MGI
Primary source	MGI:MGI:109168
See related	Ensembl:ENSMUSG00000070047
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2310038E12Rik, AU023433, Fath, mFat1
Expression	Ubiquitous expression in limb E14.5 (RPKM 20.3), colon adult (RPKM 15.5) and 24 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

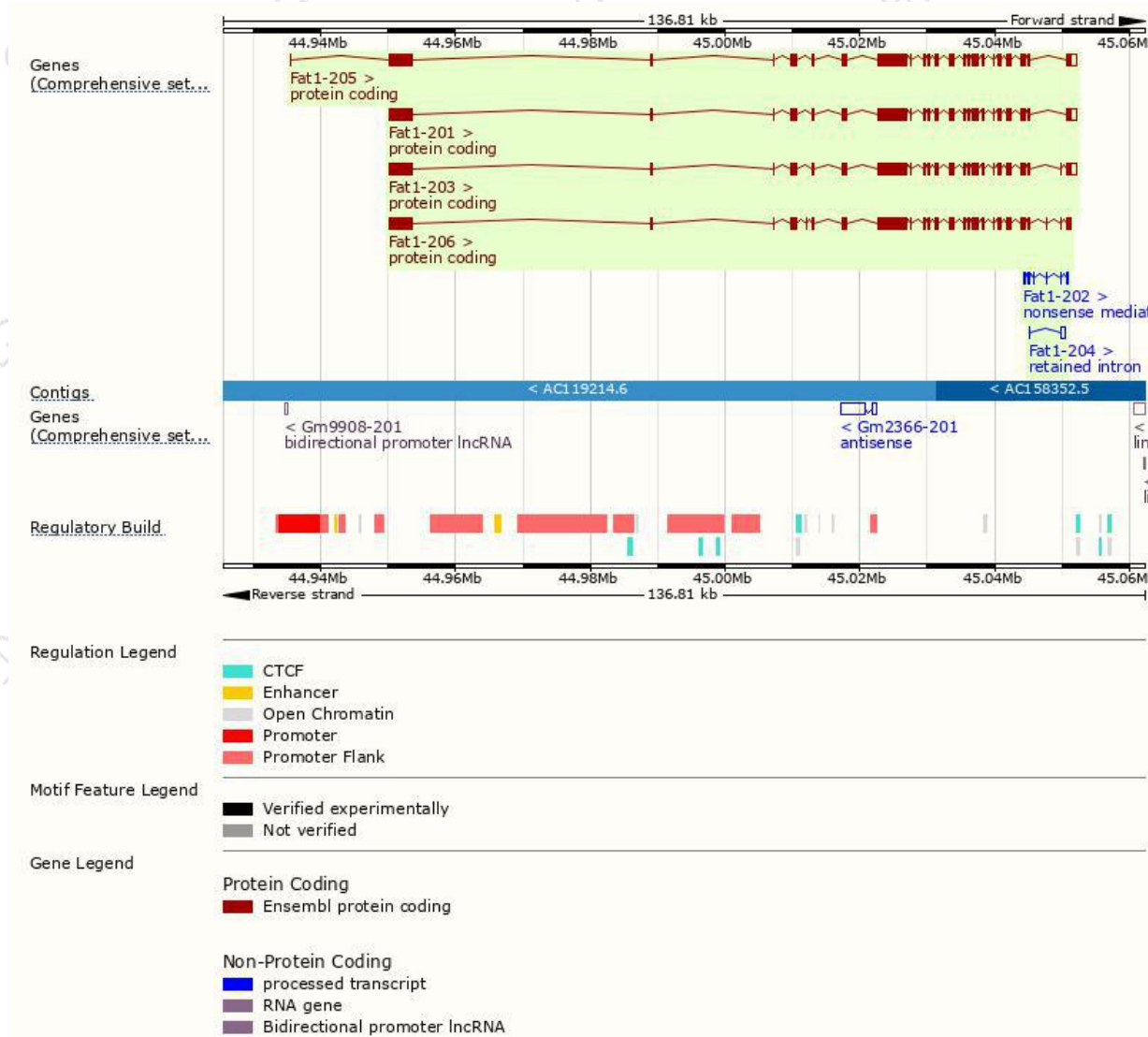
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fat1-205	ENSMUST00000191428.6	14814	4590aa	Protein coding	CCDS52547	F2Z4A3	TSL:5 GENCODE basic APPRIS P1
Fat1-201	ENSMUST00000098796.9	14627	4590aa	Protein coding	CCDS52547	F2Z4A3	TSL:5 GENCODE basic APPRIS P1
Fat1-203	ENSMUST00000189017.7	14651	4602aa	Protein coding	-	A0A087WRT4	TSL:5 GENCODE basic
Fat1-206	ENSMUST00000215588.1	13938	4645aa	Protein coding	-	A0A1L1SQU7	TSL:5 GENCODE basic
Fat1-202	ENSMUST00000186342.2	739	159aa	Nonsense mediated decay	-	A0A087WPU4	CDS 5' incomplete TSL:1
Fat1-204	ENSMUST00000189367.1	693	No protein	Retained intron	-	-	TSL:3

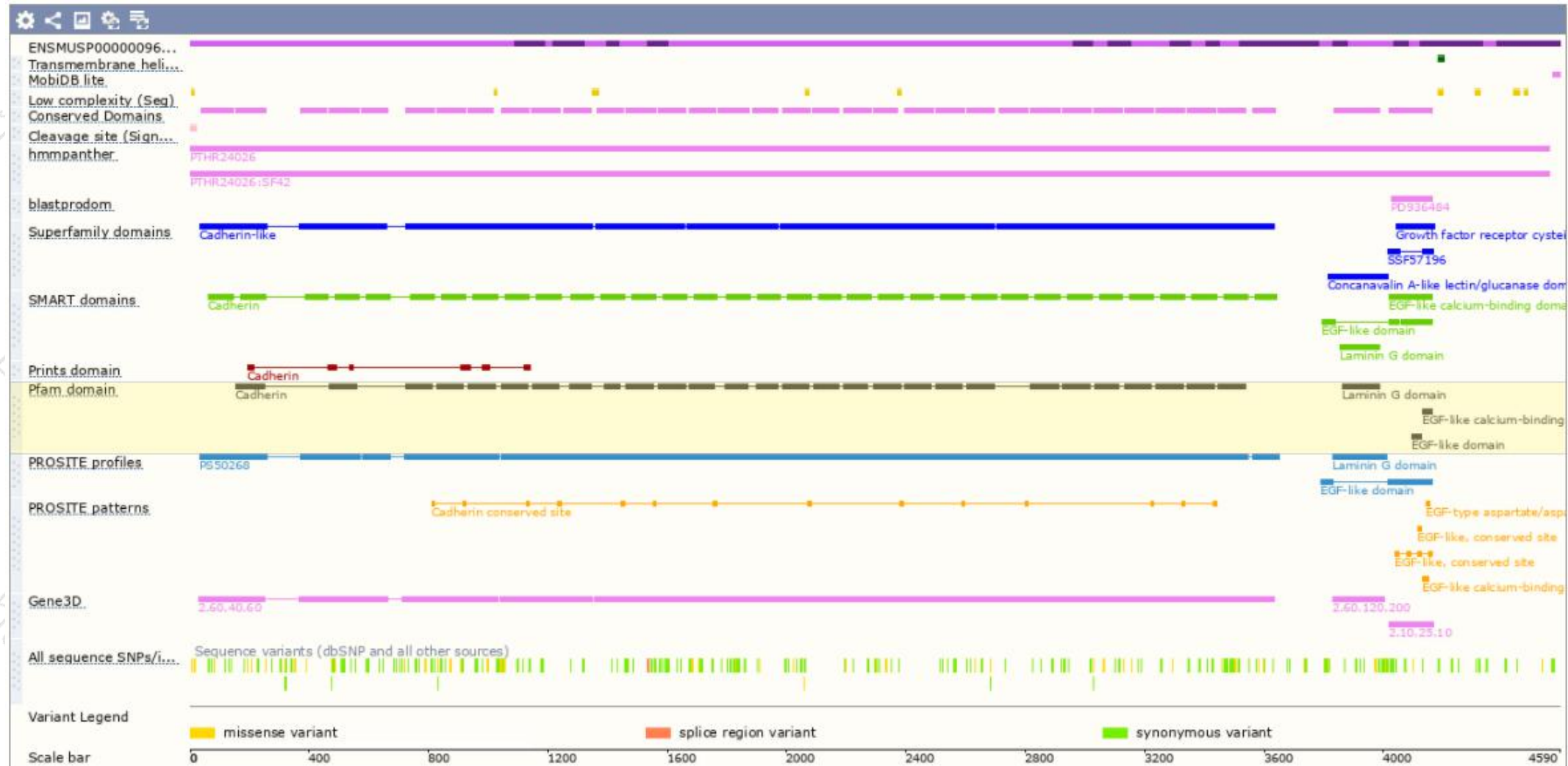
The strategy is based on the design of *Fat1-201* transcript,The transcription is shown below



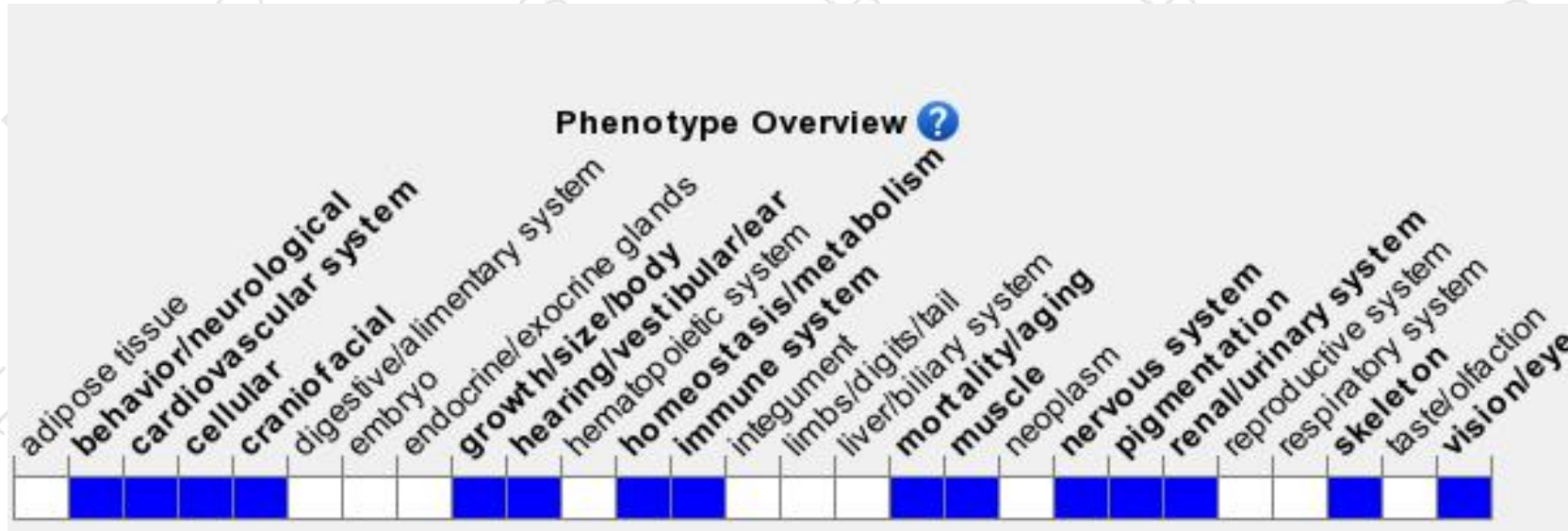
Genomic location distribution



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, Homozygotes for a targeted null mutation exhibit holoprosencephaly, anophthalmia, kidney defects and perinatal lethality. Mice homozygous for a hypomorphic allele exhibit altered shoulder girdle and facial musculature, retinal defects, abnormal inner ear patterning and kidney defects.

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

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