

Cflar Cas9-CKO Strategy

Designer:

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Design Date:

2019-8-8

Project Overview

Project Name

Cflar

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

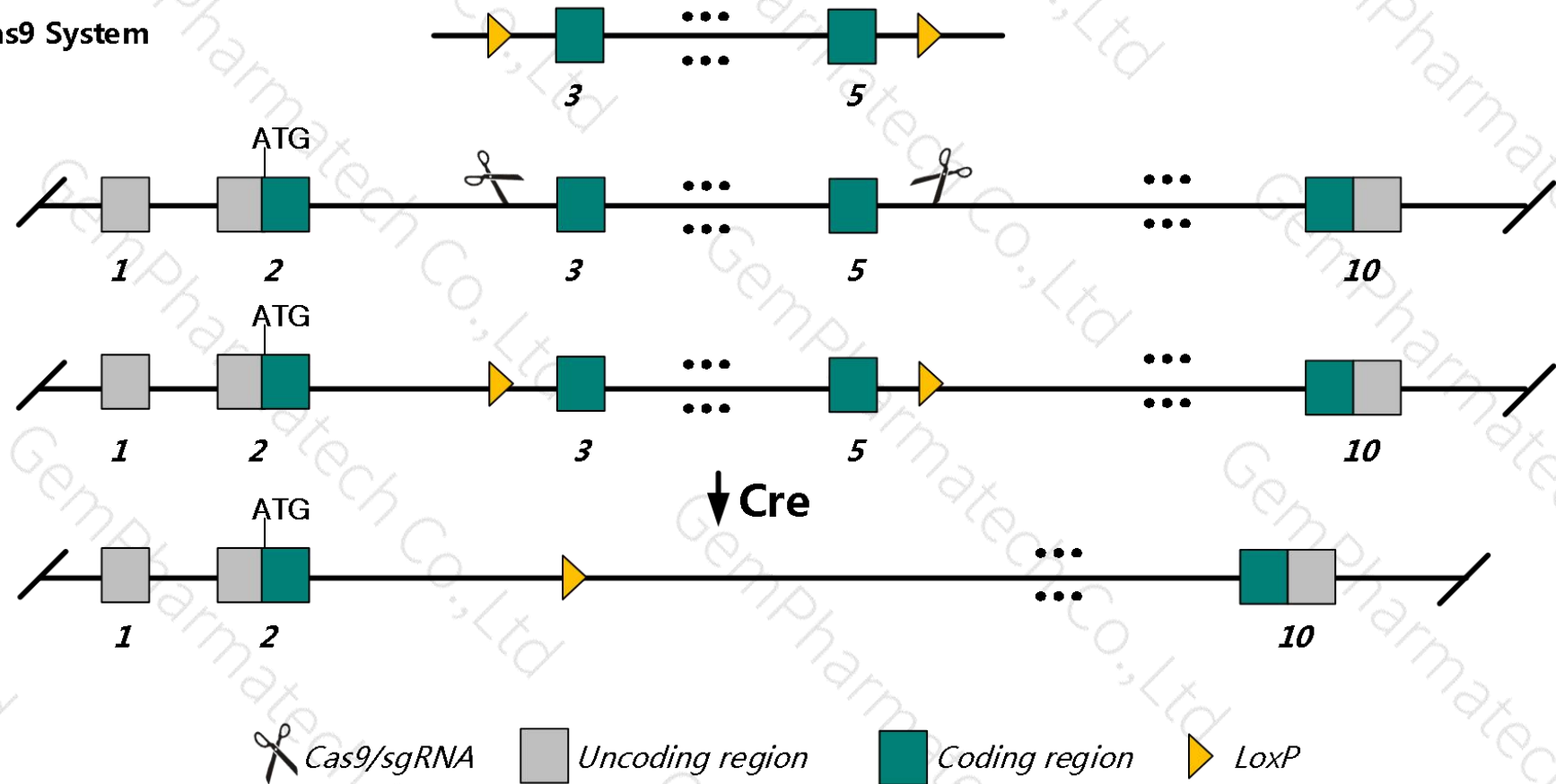
This model will use CRISPR/Cas9 technology to edit the *Cflargene*. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



Technical routes

- The *Cflargene* has 13 transcripts. According to the structure of *Cflar* gene, exon3-5 of *Cdkn2a-205* (ENSMUST00000114313.7) transcript is recommended as the knockout region. The region contains 316bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cflargene*. 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, Homozygous mutation of this gene results in embryonic lethality by E10.5. Mutant embryos exhibit cardiac developmental abnormalities and pooling of blood in the head and abdominal regions.
- The *Cflargene* is located on the Chr4. 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)

Cflar CASP8 and FADD-like apoptosis regulator [*Mus musculus* (house mouse)]

Gene ID: 12633, updated on 12-Aug-2018

Summary

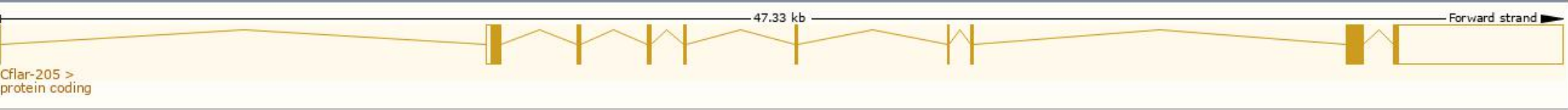
Official Symbol	Cflar provided by MGI
Official Full Name	CASP8 and FADD-like apoptosis regulator provided by MGI
Primary source	MGI:MGI:1336166
See related	Ensembl:ENSMUSG00000026031 Vega:OTTMUSG00000019581
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cash; Flip; MRIT; CLARP; FLAME; Casper; Gm9845; c-Flip; FLAME-1; I-FLICE; 2310024N18Rik; A430105C05Rik
Expression	Ubiquitous expression in lung adult (RPKM 9.2), kidney adult (RPKM 7.4) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

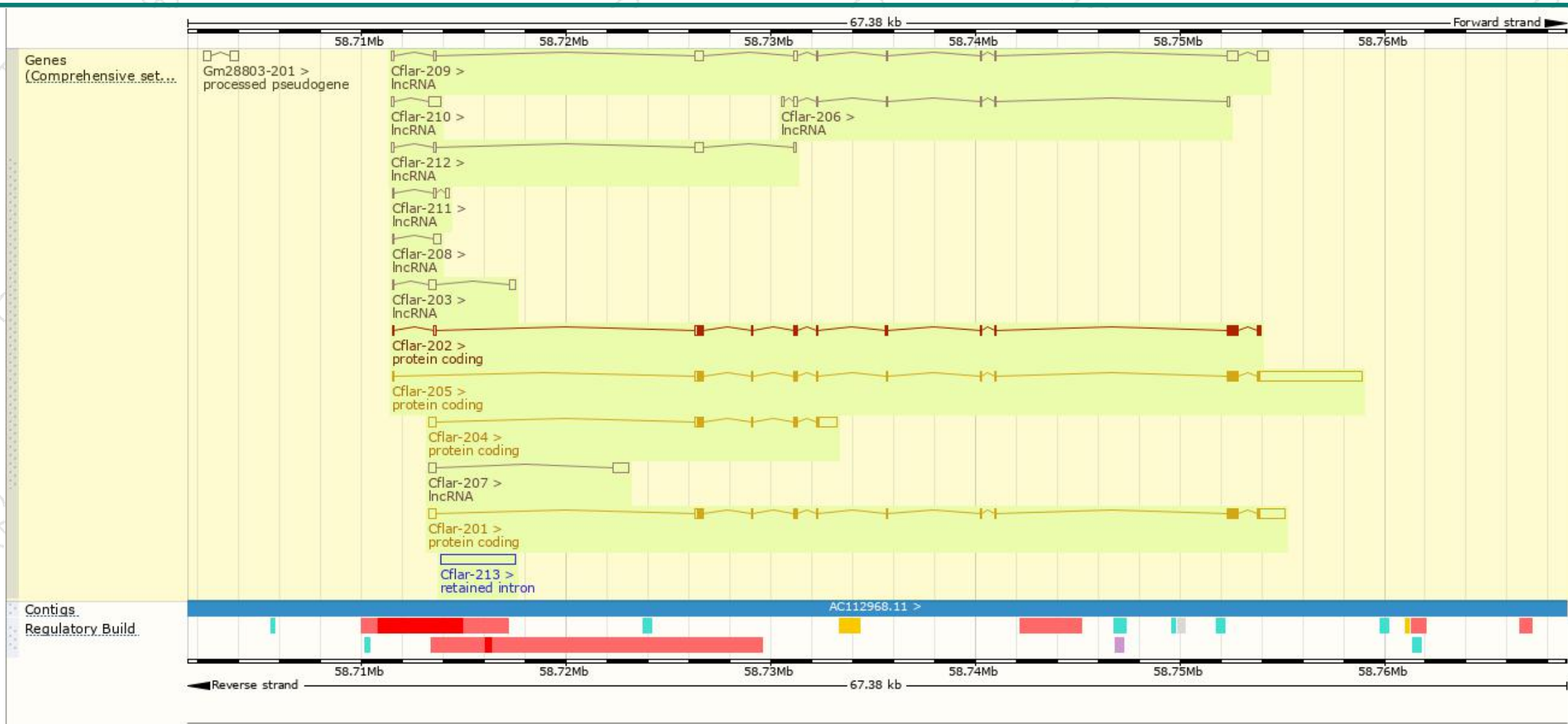
The gene has 13 transcripts,all transcripts are shown below:

Show/hide columns (1 hidden)		Filter						
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Cflar-205	ENSMUST00000114313.7	6572	481aa	Protein coding	CCDS14978	Q35732 Q812G4	NM_001289704 NM_001293804 NM_001293805 NP_001276633 NP_001280733 NP_001280734	TSL:1 GENCODE basic APPRIS P2
Cflar-201	ENSMUST00000069333.7	3163	481aa	Protein coding	CCDS14978	Q35732 Q812G4	NM_207653 NP_997536	TSL:1 GENCODE basic APPRIS P2
Cflar-204	ENSMUST00000114309.7	2010	215aa	Protein coding	CCDS35582	Q35732 Q5U4G3	NM_009805 NP_033935	TSL:1 GENCODE basic
Cflar-202	ENSMUST00000097722.8	1771	484aa	Protein coding	-	A0A2C9F2D9	-	TSL:5 GENCODE basic APPRIS ALT2
Cflar-209	ENSMUST00000140940.7	2064	No protein	Processed transcript	-	-	-	TSL:1
Cflar-207	ENSMUST00000123553.1	1100	No protein	Processed transcript	-	-	-	TSL:2
Cflar-203	ENSMUST00000097723.2	708	No protein	Processed transcript	-	-	-	TSL:3
Cflar-212	ENSMUST00000164900.7	694	No protein	Processed transcript	-	-	-	TSL:3
Cflar-210	ENSMUST00000149874.7	676	No protein	Processed transcript	-	-	NR_149258	TSL:1
Cflar-206	ENSMUST00000123032.1	623	No protein	Processed transcript	-	-	-	TSL:5
Cflar-208	ENSMUST00000124567.1	378	No protein	Processed transcript	-	-	-	TSL:2
Cflar-211	ENSMUST00000156870.1	351	No protein	Processed transcript	-	-	NR_149256	TSL:3
Cflar-213	ENSMUST00000189858.1	3677	No protein	Retained intron	-	-	-	TSL:NA

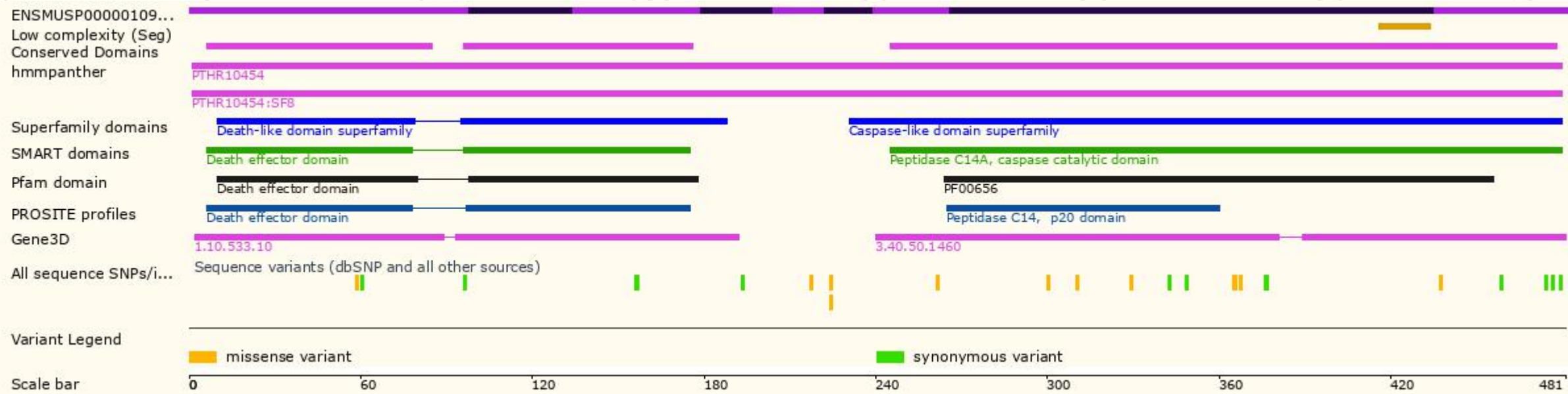
The strategy is based on the design of *Cflar-205* transcript,The transcription is shown below



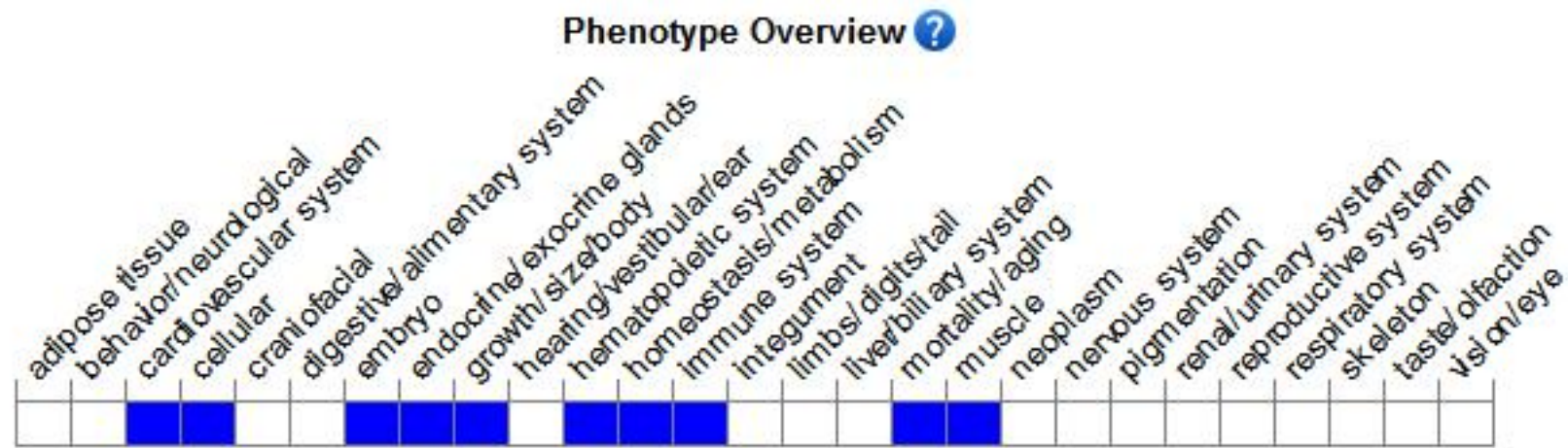
Genomic location distribution



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, Homozygous mutation of this gene results in embryonic lethality by E10.5.

Mutant embryos exhibit cardiac developmental abnormalities and pooling of blood in the head and abdominal regions.

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

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