Fryl Cas9-CKO Strategy

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Design Date: 2020-4-8

Project Overview

Project Name	***Fryl***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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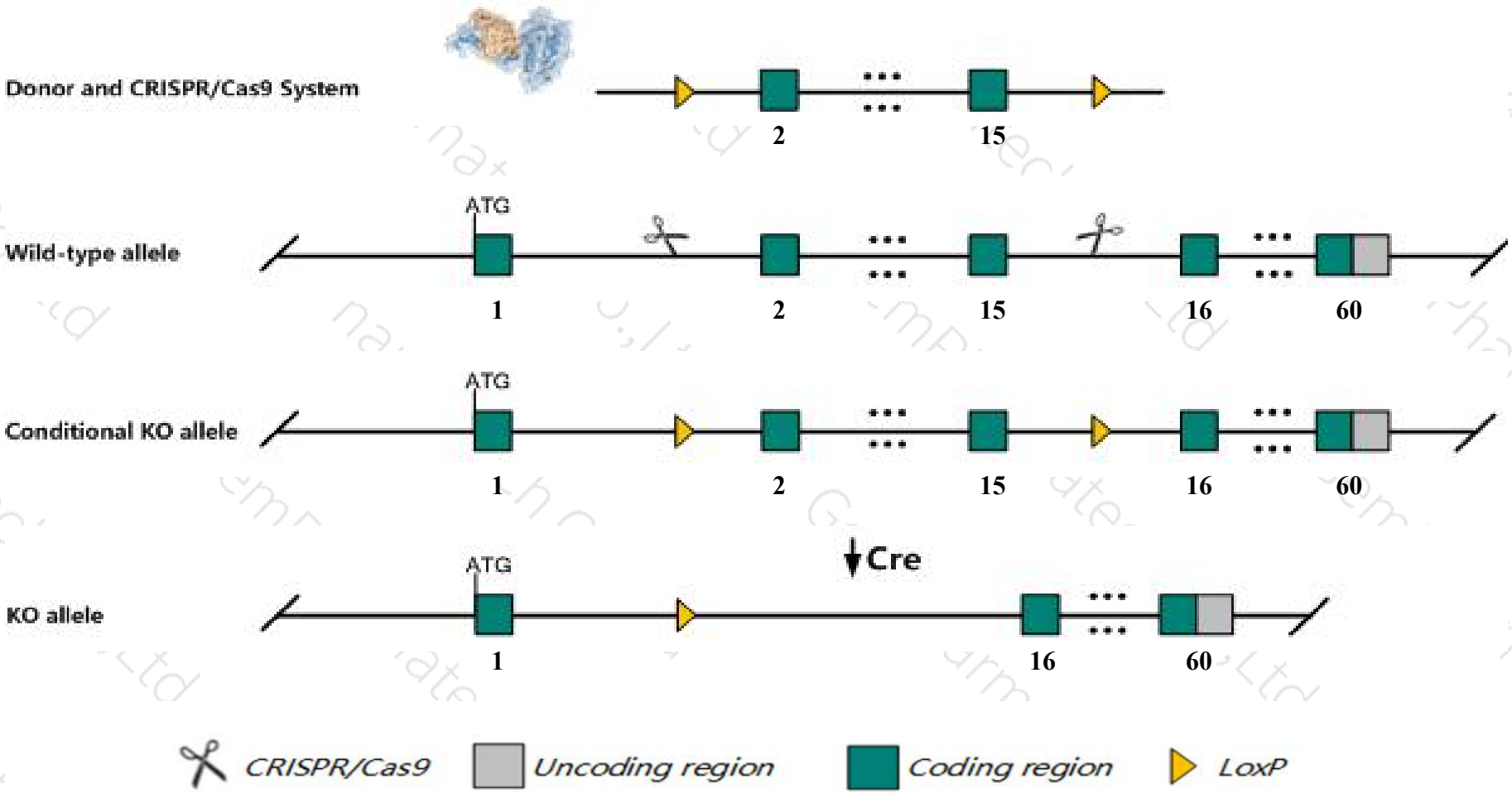
This model will use CRISPR/Cas9 technology to edit the *Fryl* gene. The schematic diagram is as follows:



Technical routes

- The *Fryl* gene has 20 transcripts. According to the structure of *Fryl* gene, exon2-exon15 of *Fryl-201* (ENSMUST00000094700.10) transcript is recommended as the knockout region.The region contains 1520bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Fryl* 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,Most mice homozygous for a knock-out allele exhibit postnatal lethality and defects in kidney development; rare survivors display growth retardation, decreased body weight, and premature death associated with chronic hydronephrosis.

➢ Transcript *Fryl-212,Fryl-214,Fryl-217,Fryl-219 and Fryl-220* may not be affected.

➢ The *Fryl* gene is located on the Chr5. 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）

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Fryl FRY like transcription coactivator [Mus musculus (house mouse)]

Gene ID: 72313, updated on 13-Mar-2020

Summary

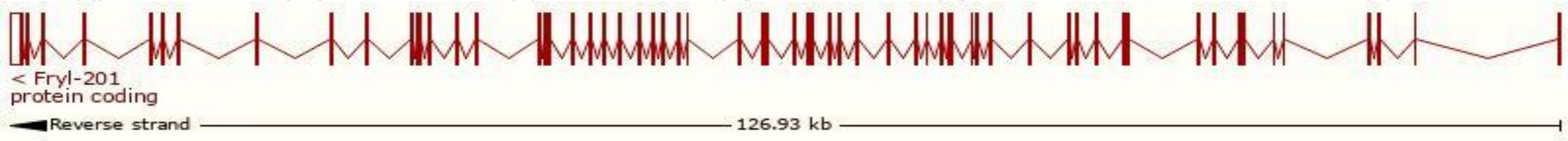
Official Symbol	Fryl provided by MGI
Official Full Name	FRY like transcription coactivator provided by MGI
Primary source	MGI:MGI:1919563
See related	Ensembl:ENSMUSG00000070733
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2010313D22Rik, 2310004H21Rik, 2510002A14Rik, 9030227G01Rik, mKIAA0826
Expression	Ubiquitous expression in thymus adult (RPKM 7.6), colon adult (RPKM 7.0) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

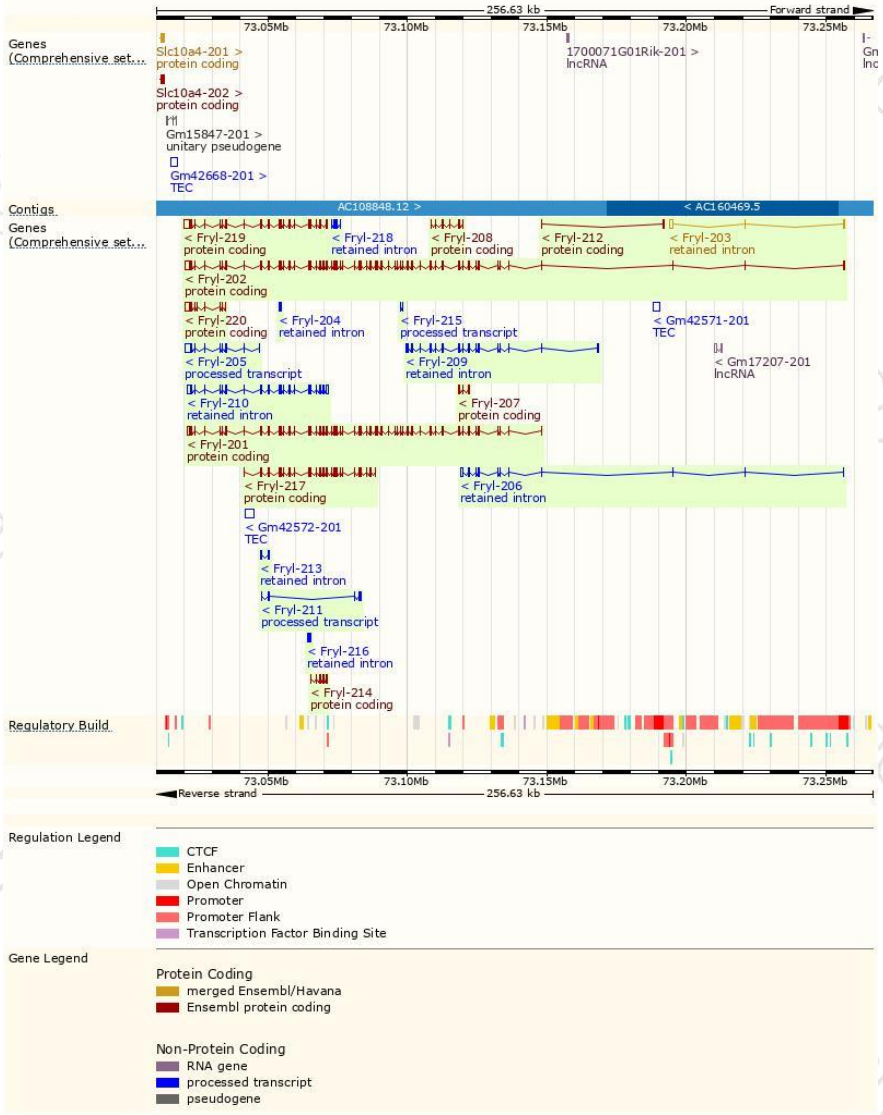
The gene has 20 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fryl-202	ENSMUST00000101127.11	11340	3007aa	Protein coding	CCDS19337	F8VQ05	TSL:5 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 P1
Fryl-201	ENSMUST00000094700.10	9852	3007aa	Protein coding	CCDS19337	F8VQ05	TSL:2 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 P1
Fryl-219	ENSMUST00000202697.3	5743	1230aa	Protein coding	-	A0A0J9YTS1	CDS 5' incomplete TSL:5
Fryl-217	ENSMUST00000202381.3	4392	1464aa	Protein coding	-	A0A0J9YUH4	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Fryl-220	ENSMUST00000202806.3	2707	331aa	Protein coding	-	A0A0J9YU33	CDS 5' incomplete TSL:5
Fryl-214	ENSMUST00000201277.1	723	241aa	Protein coding	-	A0A0J9YUJ6	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Fryl-208	ENSMUST00000153903.2	600	200aa	Protein coding	-	F6Q1V8	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Fryl-207	ENSMUST00000152631.1	495	124aa	Protein coding	-	F6SDM9	CDS 5' incomplete TSL:3
Fryl-212	ENSMUST00000176910.2	449	129aa	Protein coding	-	H3BLH9	CDS 3' incomplete TSL:3
Fryl-205	ENSMUST00000146953.7	3088	No protein	Processed transcript	-	-	TSL:1
Fryl-211	ENSMUST00000175890.1	606	No protein	Processed transcript	-	-	TSL:2
Fryl-215	ENSMUST00000201405.1	402	No protein	Processed transcript	-	-	TSL:3
Fryl-210	ENSMUST00000156661.8	5056	No protein	Retained intron	-	-	TSL:1
Fryl-209	ENSMUST00000153923.7	3007	No protein	Retained intron	-	-	TSL:2
Fryl-206	ENSMUST00000148433.7	1899	No protein	Retained intron	-	-	TSL:1
Fryl-203	ENSMUST00000123446.2	1641	No protein	Retained intron	-	-	TSL:2
Fryl-216	ENSMUST00000201841.1	543	No protein	Retained intron	-	-	TSL:2
Fryl-204	ENSMUST00000143665.2	520	No protein	Retained intron	-	-	TSL:1
Fryl-218	ENSMUST00000202413.1	404	No protein	Retained intron	-	-	TSL:3
Fryl-213	ENSMUST00000201200.1	376	No protein	Retained intron	-	-	TSL:3

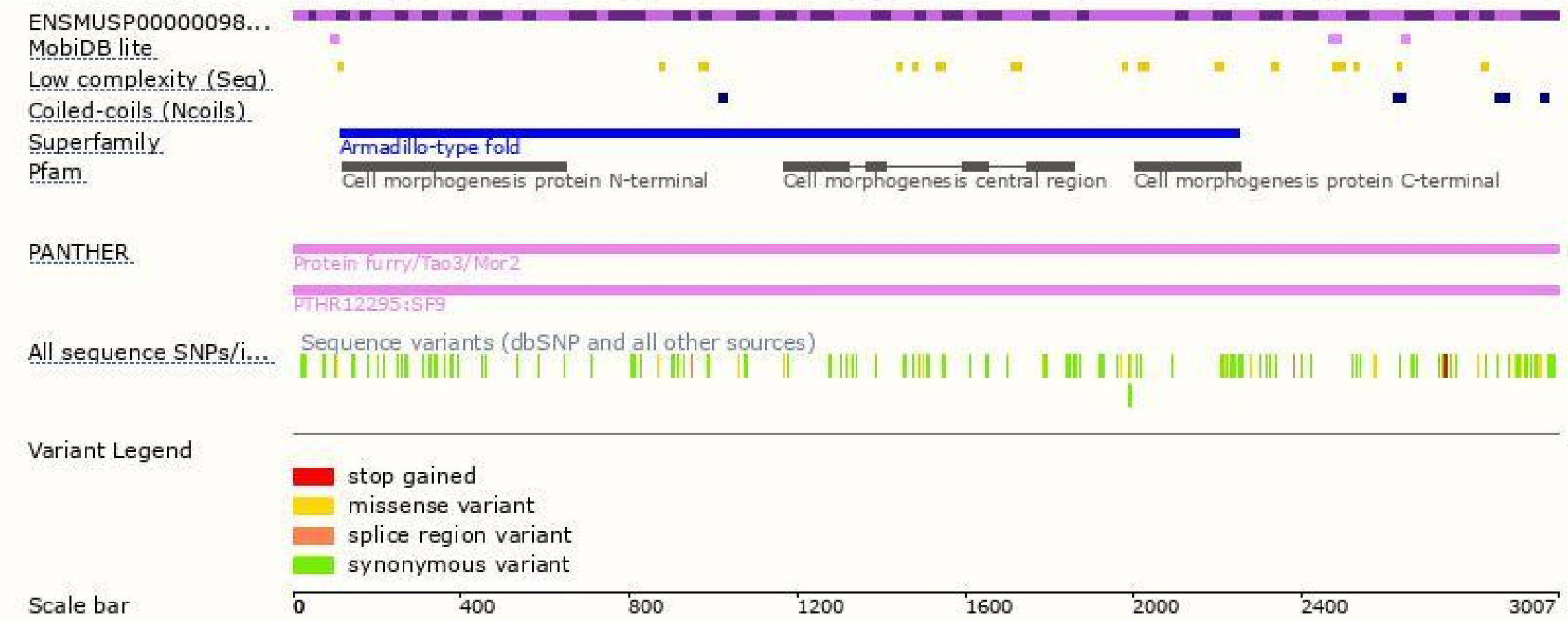
The strategy is based on the design of *Fryl-201* transcript,The transcription is shown below



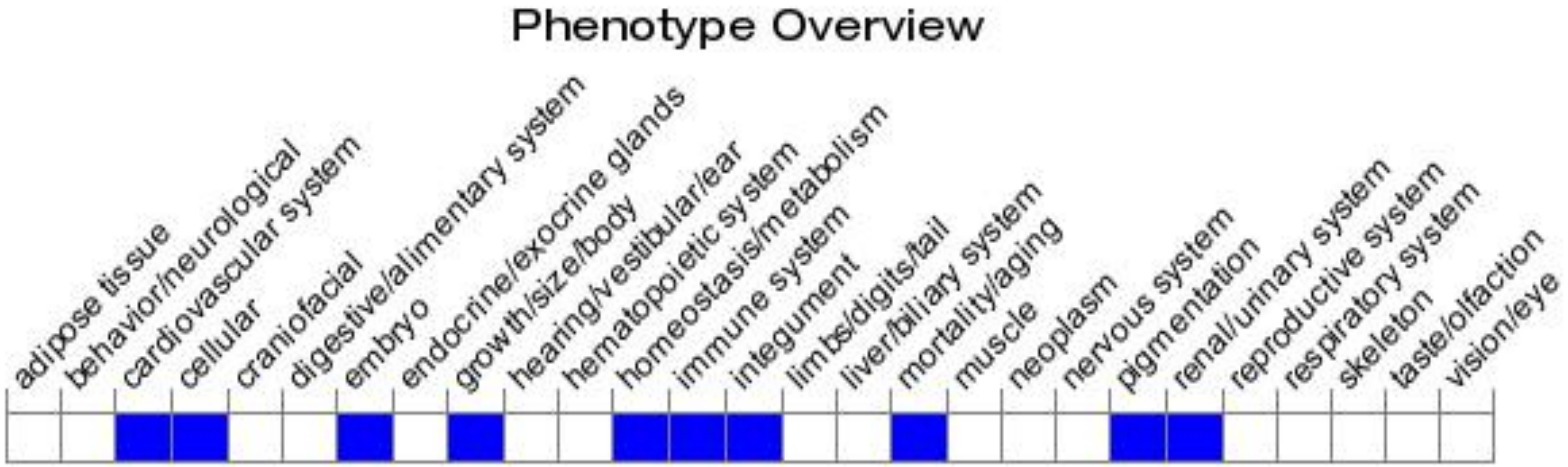
Genomic location distribution



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,Most mice homozygous for a knock-out allele exhibit postnatal lethality and defects in kidney development; rare survivors display growth retardation, decreased body weight, and premature death associa with chronic hydronephrosis.

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

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