Txnrd2 Cas9-CKO Strategy

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Project Overview

Project Name	***Txnrd2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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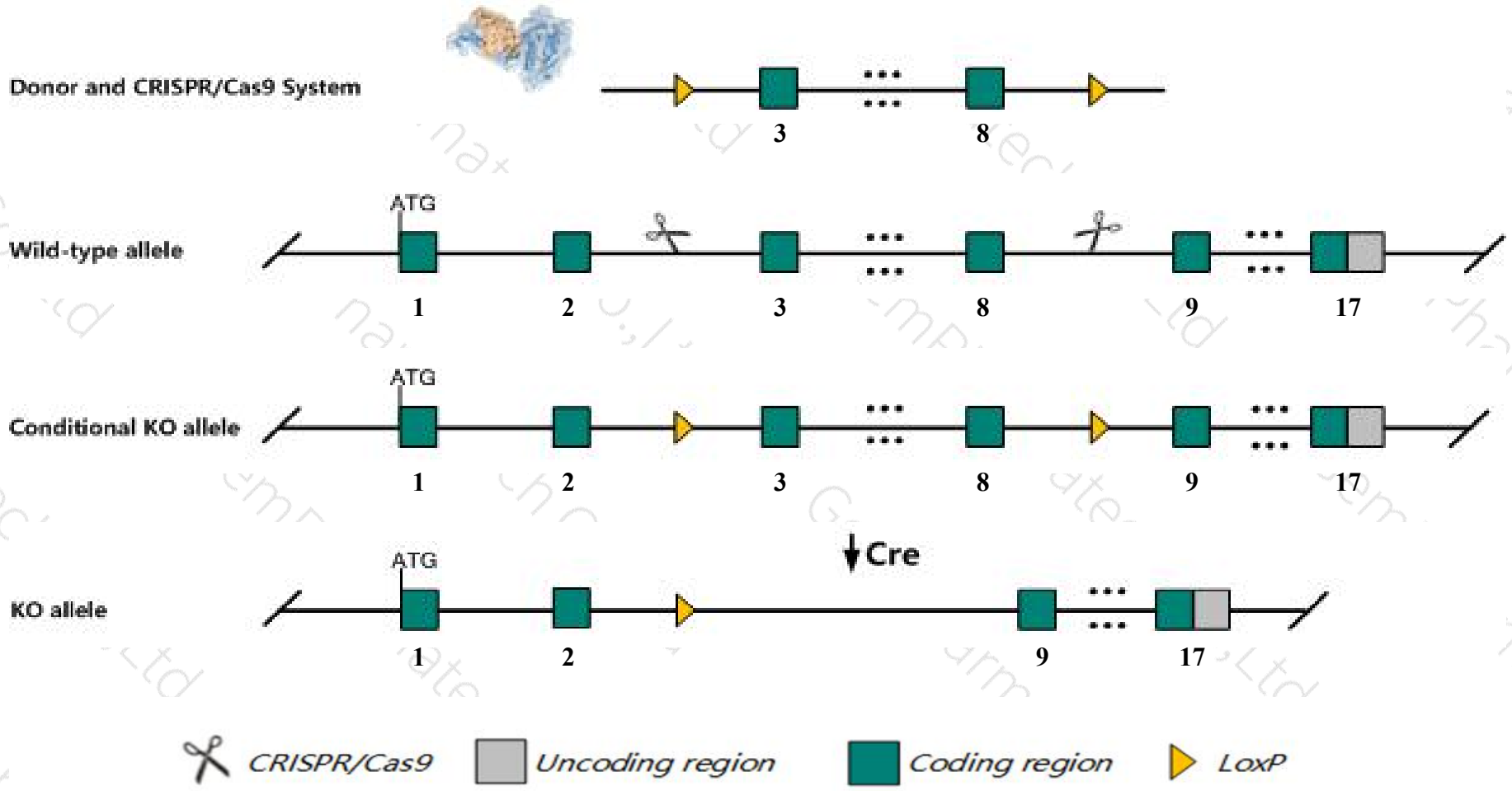
This model will use CRISPR/Cas9 technology to edit the *Txnrd2* gene. The schematic diagram is as follows:



Technical routes

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- The *Txnrd2* gene has 16 transcripts. According to the structure of *Txnrd2* gene, exon3-exon8 of *Txnrd2-215* (ENSMUST00000206151.1) transcript is recommended as the knockout region.The region contains 490bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Txnrd2* 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,mice homozygous for a knock-out allele die at e13 due to severe anemia and growth retardation, resulting from perturbed cardiac development and augmented apoptosis of hematopoietic cells.
- The effect on transcript *Txnrd2*-207 is unknown.
- Transcript *Txnrd2*-204&209&210&211 may not be affected.
- The *Txnrd2* gene is located on the Chr16. 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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Txnrd2 thioredoxin reductase 2 [Mus musculus (house mouse)]

Gene ID: 26462, updated on 13-Mar-2020

Summary

Official Symbol	Txnrd2 provided by MGI
Official Full Name	thioredoxin reductase 2 provided byMGI
Primary source	MGI:MGI:1347023
See related	Ensembl:ENSMUSG00000075704
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA118373, ESTM573010, TGR, Tr3, Trxr2, Trxrd2
Summary	The protein encoded by this gene belongs to the pyridine nucleotide-disulfide oxidoreductase family, and is a member of the thioredoxin (Trx) system. Three thioredoxin reductase (TrxR) isozymes are found in mammals. TrxRs are selenocysteine-containing flavoenzymes, which reduce thioredoxins, as well as other substrates, and play a key role in redox homoeostasis. This gene encodes a mitochondrial form important for scavenging reactive oxygen species in mitochondria. It functions as a homodimer containing FAD, and selenocysteine (Sec) at the active site. Sec is encoded by UGA codon that normally signals translation termination. The 3' UTRs of selenoprotein mRNAs contain a conserved stem-loop structure, the Sec insertion sequence (SECIS) element, which is necessary for the recognition of UGA as a Sec codon rather than as a stop signal. Alternatively spliced transcript variants encoding different isoforms have been described for this gene. [provided by RefSeq, Jun 2017]
Expression	Ubiquitous expression in liver E14.5 (RPKM 41.1), liver E14 (RPKM 39.1) and 27 other tissuesSee more
Orthologs	human all

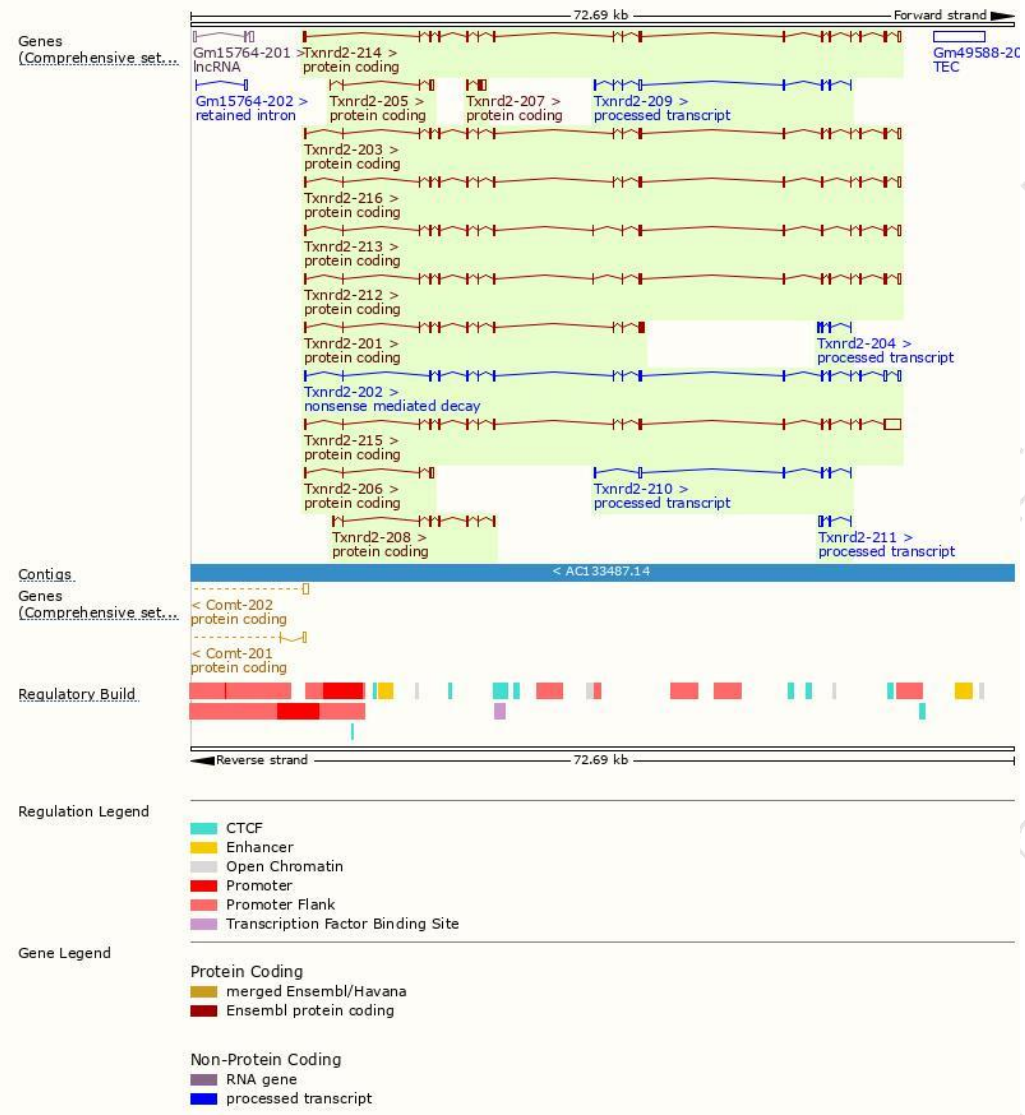
Transcript information（Ensembl）

The gene has 16 transcripts,all transcripts are shown below:

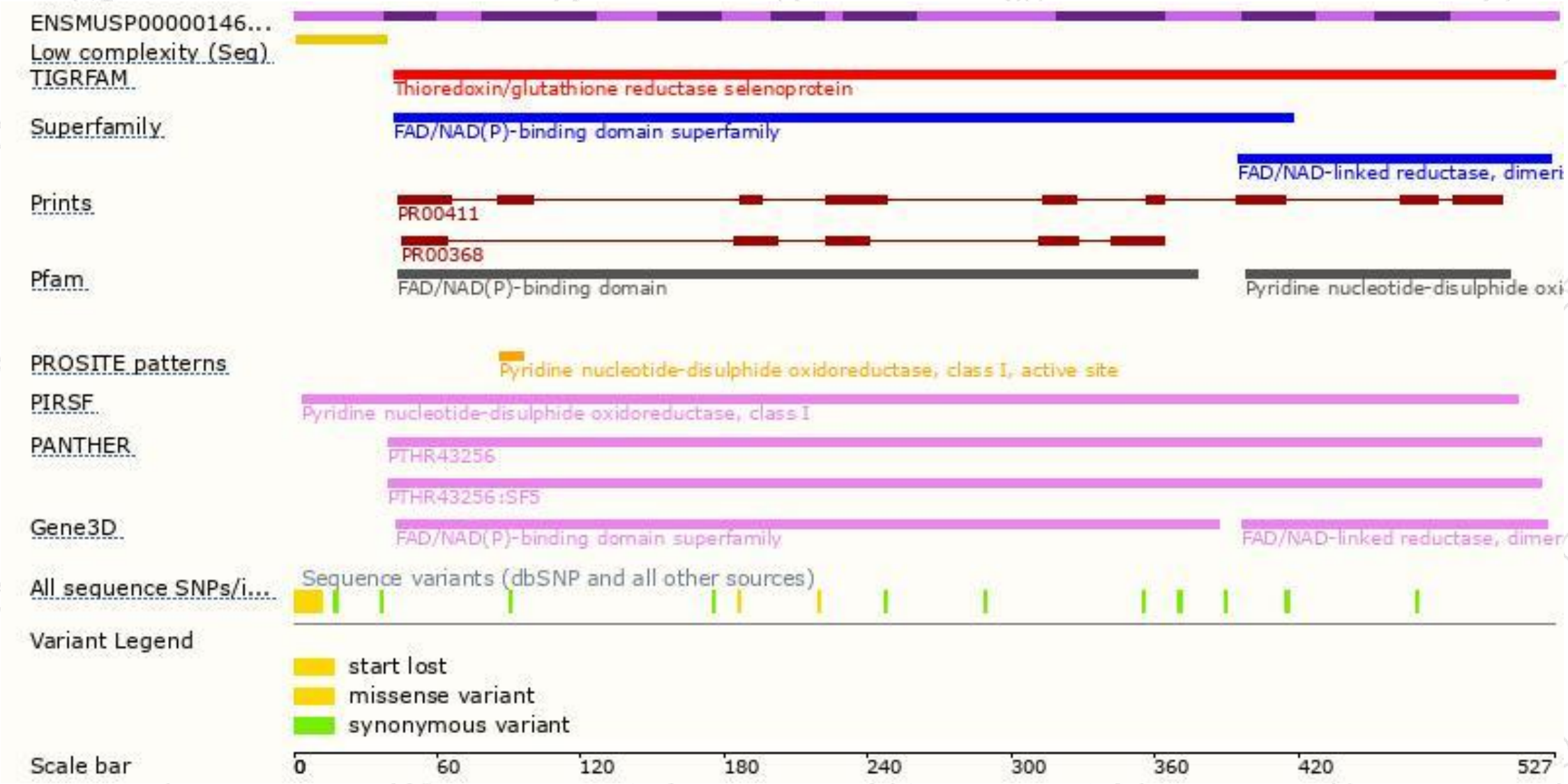
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Txnrd2-215	ENSMUST00000206151.1	2874	527aa	Protein coding	CCDS79434	A0A0M3HEQ0	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Txnrd2-203	ENSMUST00000115606.7	1905	527aa	Protein coding	CCDS79434	A0A0M3HEQ0	TSL:1 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 P2
Txnrd2-212	ENSMUST00000177856.7	1878	522aa	Protein coding	-	J3QMQ8	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 ALT2
Txnrd2-214	ENSMUST00000205679.1	1866	505aa	Protein coding	-	A0A0U1RQA4	TSL:5 GENCODE basic
Txnrd2-216	ENSMUST00000206606.1	1809	496aa	Protein coding	-	A0A0U1RPV8	TSL:1 GENCODE basic
Txnrd2-213	ENSMUST00000178093.2	1785	491aa	Protein coding	-	J3QMN4	TSL:5 GENCODE basic
Txnrd2-201	ENSMUST00000115604.7	1235	359aa	Protein coding	-	A0A0M3HEP9	TSL:1 GENCODE basic
Txnrd2-207	ENSMUST00000138310.2	640	96aa	Protein coding	-	A0A0U1RPY5	CDS 5' incomplete TSL:3
Txnrd2-208	ENSMUST00000144233.2	593	165aa	Protein coding	-	A0A0U1RPC6	CDS 3' incomplete TSL:5
Txnrd2-206	ENSMUST00000131303.7	532	123aa	Protein coding	-	A0A0U1RPS1	CDS 5' incomplete TSL:2
Txnrd2-205	ENSMUST00000126778.7	491	101aa	Protein coding	-	Q9D8I4	TSL:1 GENCODE basic
Txnrd2-202	ENSMUST00000115605.7	1800	466aa	Nonsense mediated decay	-	D3Z0K8	TSL:5
Txnrd2-209	ENSMUST00000149937.7	720	No protein	Processed transcript	-	-	TSL:5
Txnrd2-210	ENSMUST00000153457.7	648	No protein	Processed transcript	-	-	TSL:5
Txnrd2-211	ENSMUST00000156604.2	403	No protein	Processed transcript	-	-	TSL:5
Txnrd2-204	ENSMUST00000126330.7	266	No protein	Processed transcript	-	-	TSL:5

The strategy is based on the design of *Txnrd2-215* transcript,the transcription is shown below:

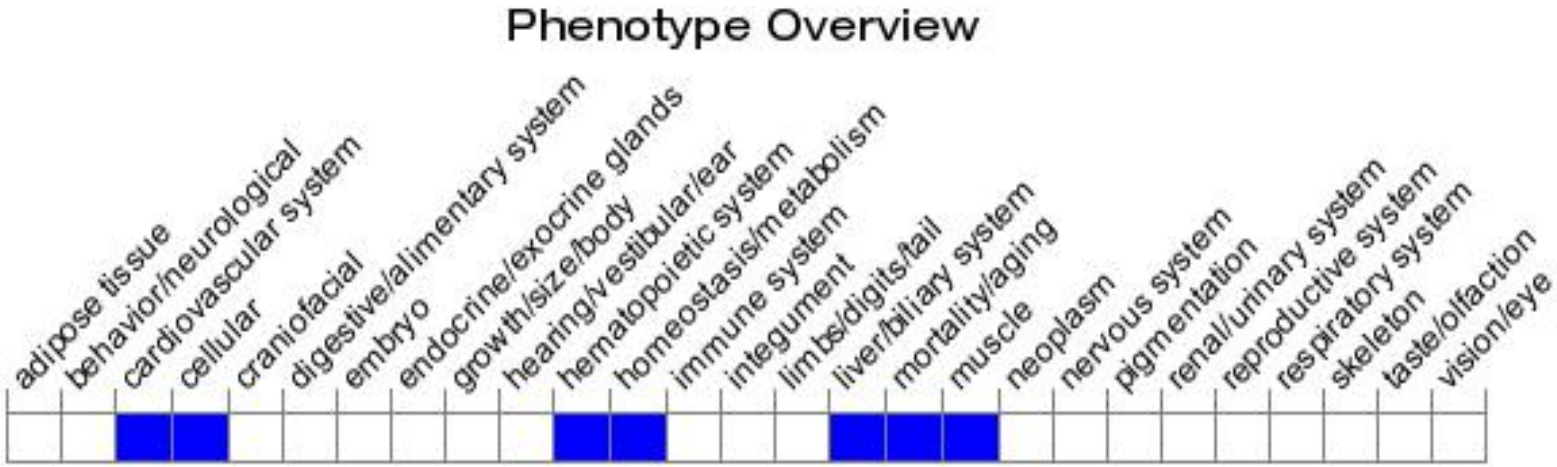
Genomic location distribution



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 die at E13 due to severe anemia and growth retardation, resulting from perturbed cardiac development and augmented apoptosis of hematopoietic cells.

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