Lig1 Cas9-CKO *Strategy*

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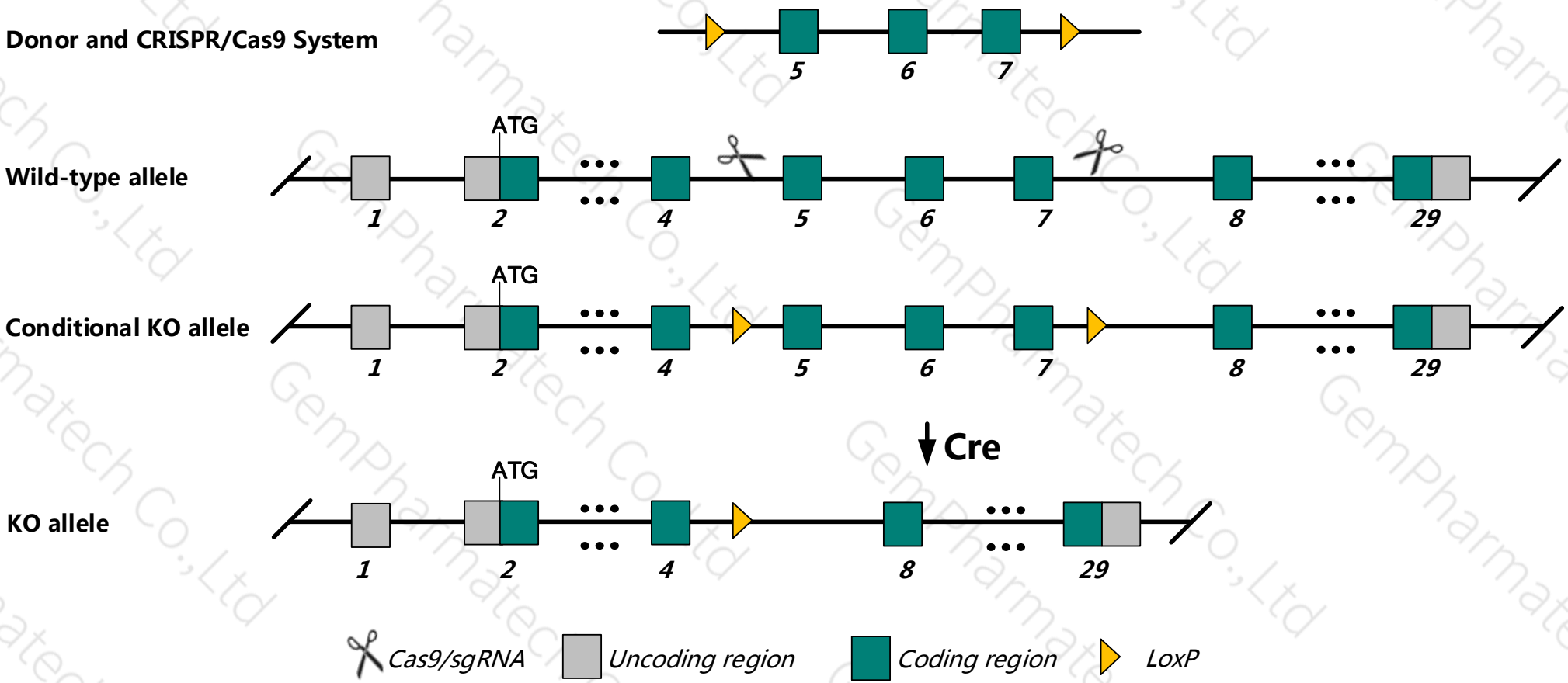
Project Overview

Project Name	***Lig1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lig1* gene. The schematic diagram is as follows:



Technical routes

- The *Lig1* gene has 12 transcripts.According to the structure of *Lig1* gene, exon5~exon7 of *Lig1*-211 （ENSMUST00000177588.7)transcript is recommended as the knockout region.The region contains 325bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Lig1* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

Notice

- According to the existing MGI data，mice homozygous for a null allele exhibit impaired fetal hematopoiesis, develop anemia, and die by E16.5.
- The *Lig1* gene is located on the Chr7. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

Lig1 ligase I, DNA, ATP-dependent [*Mus musculus* (house mouse)]

Gene ID: 16881, updated on 6-Apr-2020

Summary

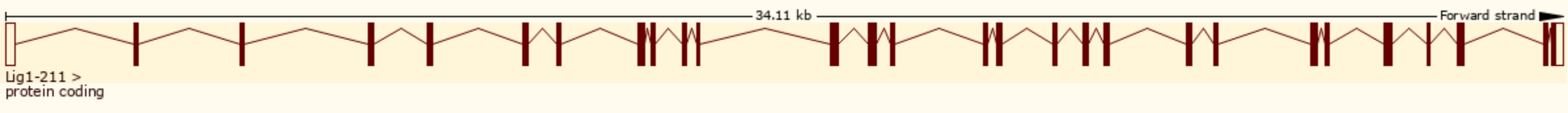
Official Symbol	Lig1 provided by MGI
Official Full Name	ligase I, DNA, ATP-dependent provided by MGI
Primary source	MGI:MGI:101789
See related	Ensembl:ENSMUSG00000056394
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Ligl; AL033288
Expression	Broad expression in liver E14 (RPKM 34.7), liver E14.5 (RPKM 31.4) and 22 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

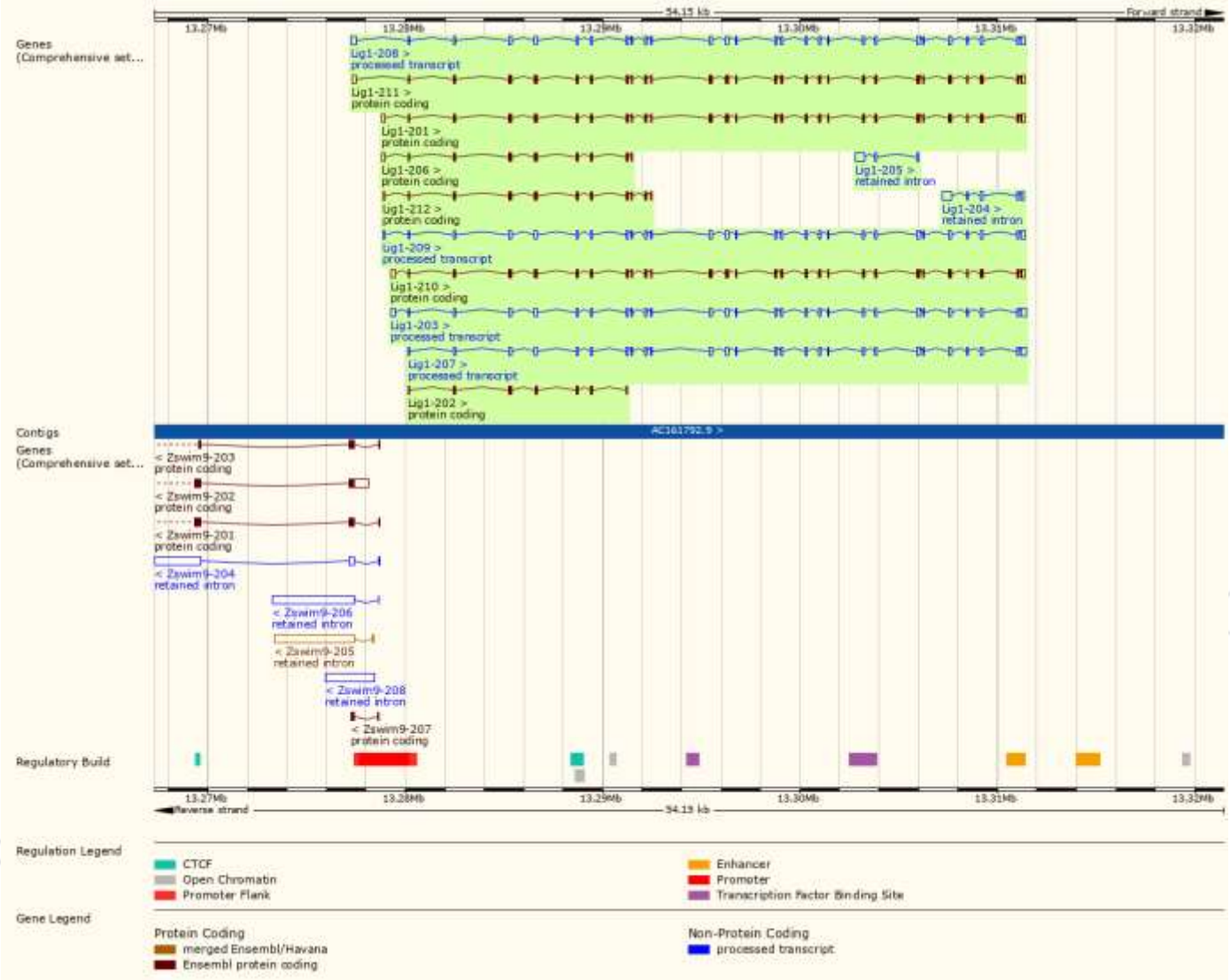
The gene has 12 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lig1-204	ENSMUST00000131294.2	946	No protein	Retained intron	-	-	TSL:1
Lig1-205	ENSMUST00000138453.1	657	No protein	Retained intron	-	-	TSL:2
Lig1-211	ENSMUST00000177588.7	3174	932aa	Protein coding	-	Q3U4X8	TSL:5 GENCODE basic APPRIS P1
Lig1-210	ENSMUST00000165964.7	3166	932aa	Protein coding	-	Q3U4X8	TSL:5 GENCODE basic APPRIS P1
Lig1-201	ENSMUST00000098814.10	3091	932aa	Protein coding	-	Q3U4X8	TSL:5 GENCODE basic APPRIS P1
Lig1-212	ENSMUST00000185145.7	1053	313aa	Protein coding	-	V9GWZ4	CDS 3' incomplete TSL:5
Lig1-206	ENSMUST00000146998.7	944	270aa	Protein coding	-	E9PV36	CDS 3' incomplete TSL:5
Lig1-202	ENSMUST00000123025.1	688	225aa	Protein coding	-	D3YUW4	CDS 3' incomplete TSL:3
Lig1-208	ENSMUST00000148471.8	3205	No protein	Processed transcript	-	-	TSL:1
Lig1-203	ENSMUST00000123846.8	3175	No protein	Processed transcript	-	-	TSL:1
Lig1-209	ENSMUST00000156525.8	3054	No protein	Processed transcript	-	-	TSL:1
Lig1-207	ENSMUST00000147735.8	2968	No protein	Processed transcript	-	-	TSL:1

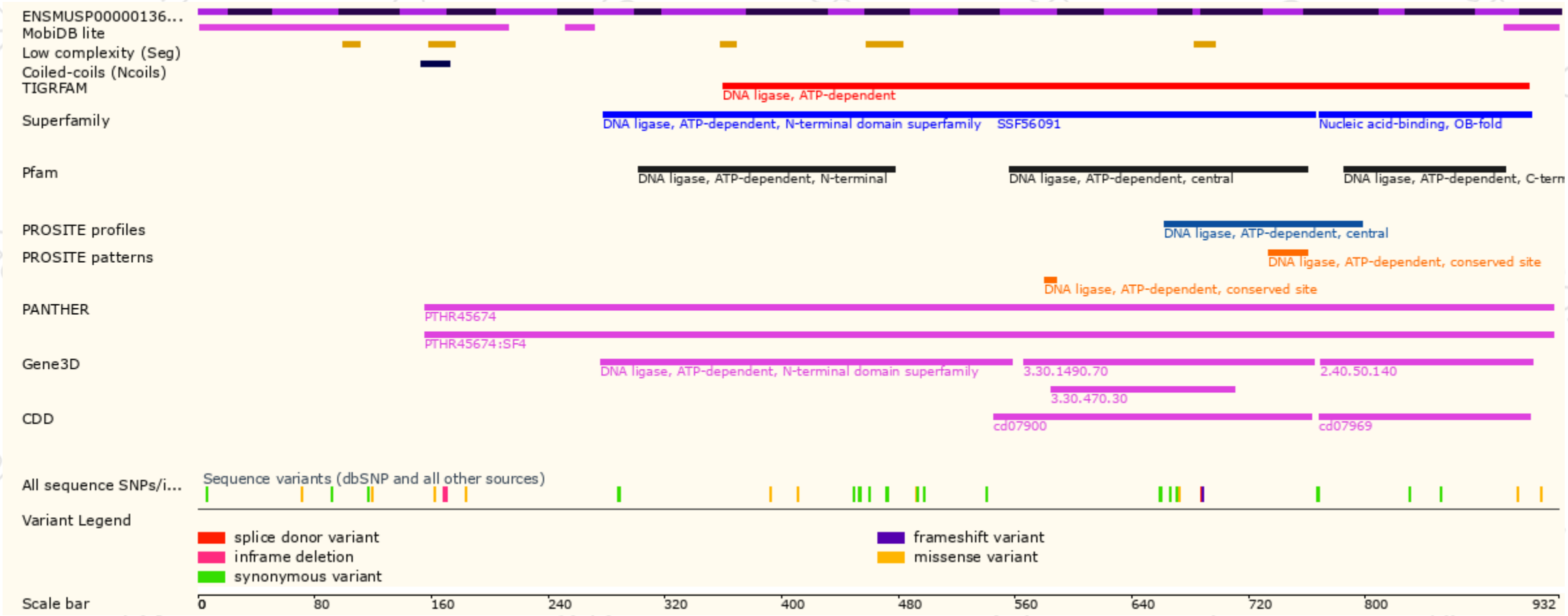
The strategy is based on the design of *Lig1*-211 transcript,the transcription is shown below:



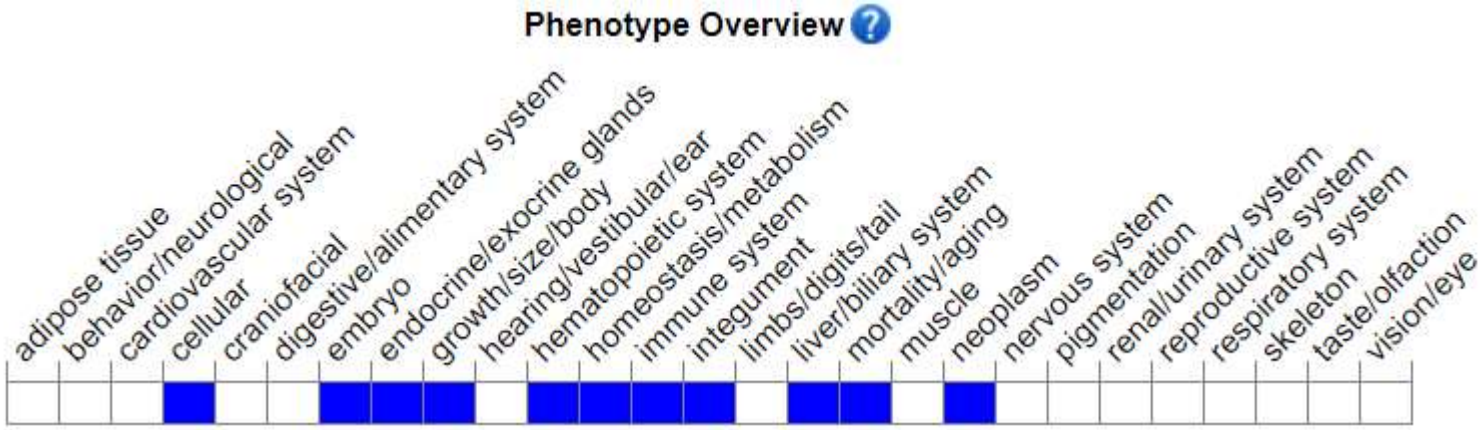
Genomic location distribution



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 null allele exhibit impaired fetal hematopoiesis, develop anemia, and die by E16.5.

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

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