Lpin1 Cas9-CKO Strategy

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Project Overview

Project Name	***Lpin1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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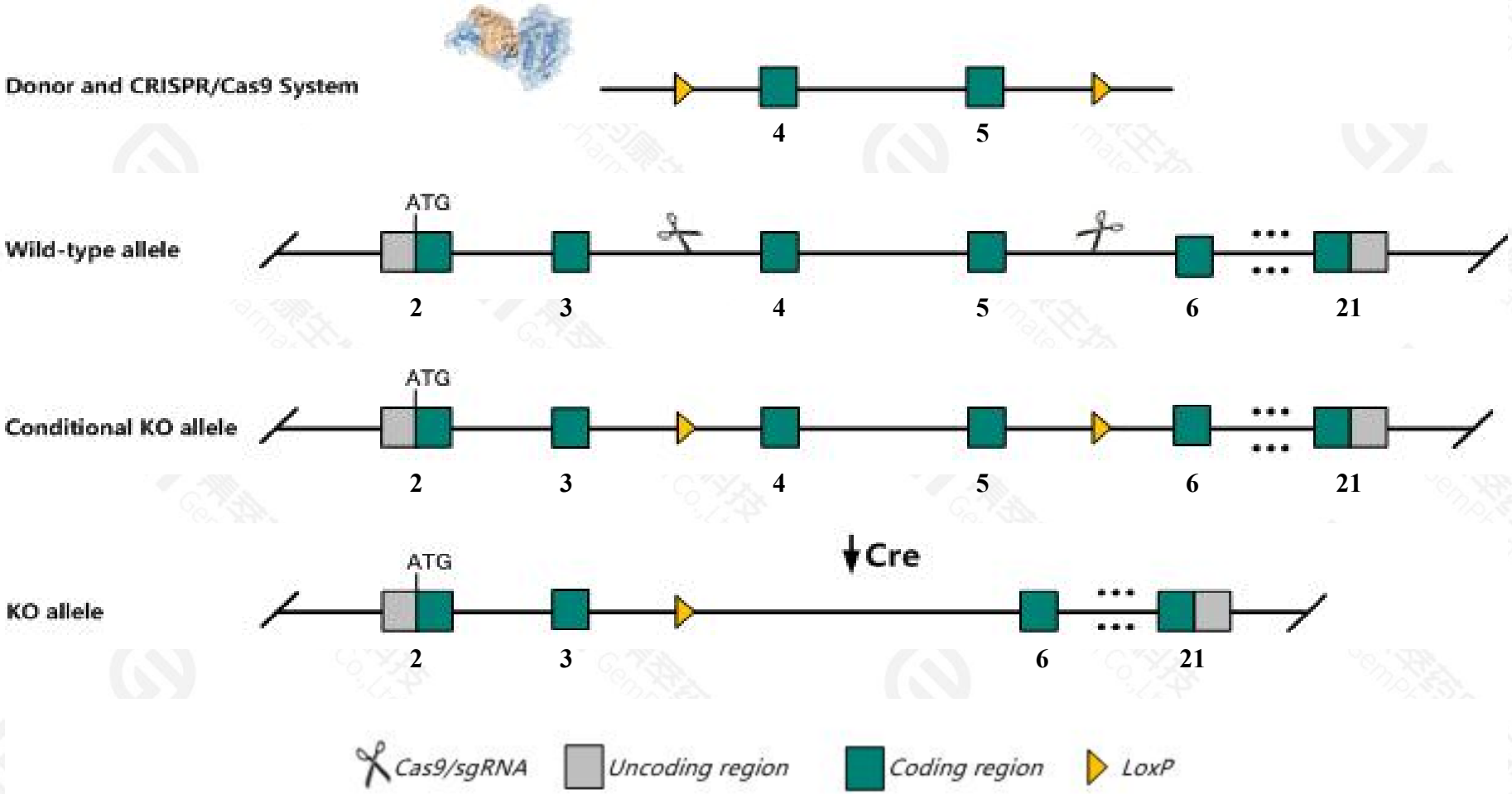
This model will use CRISPR/Cas9 technology to edit the *Lpin1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Lpin1* gene has 10 transcripts. According to the structure of *Lpin1* gene, exon4-exon5 of *Lpin1-201*(ENSMUST00000067124.5) transcript is recommended as the knockout region.The region contains 434bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Lpin1* 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 and cell types.

Notice

➢ According to the existing MGI data,eNU-induced mutants show transient hindlimb paralysis, demyelination and myelin sheath defects. Spontaneous mutants show neonatal fatty liver and hypertriglyceridemia, runting, male sterility, peripheral neuropathy, and altered hair growth, myelination, adipogenesis and lipid and glucose metabolism.

➢ The *Lpin1* gene is located on the Chr12. 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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Lpin1 lipin 1 [Mus musculus (house mouse)]

Gene ID: 14245, updated on 13-Mar-2020

Summary

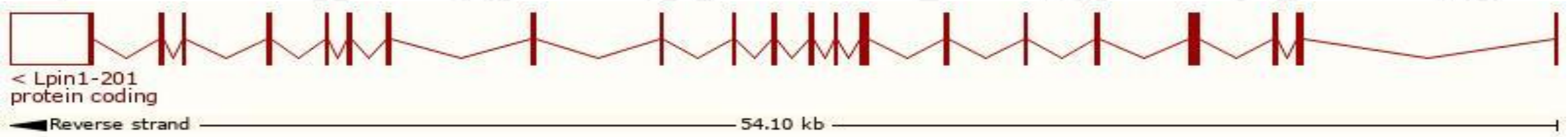
Official Symbol	Lpin1 provided by MGI
Official Full Name	lipin 1 provided by MGI
Primary source	MGI:MGI:1891340
See related	Ensembl:ENSMUSG00000020593
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Lipin1, fld
Expression	Broad expression in testis adult (RPKM 79.1), subcutaneous fat pad adult (RPKM 36.4) and 17 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

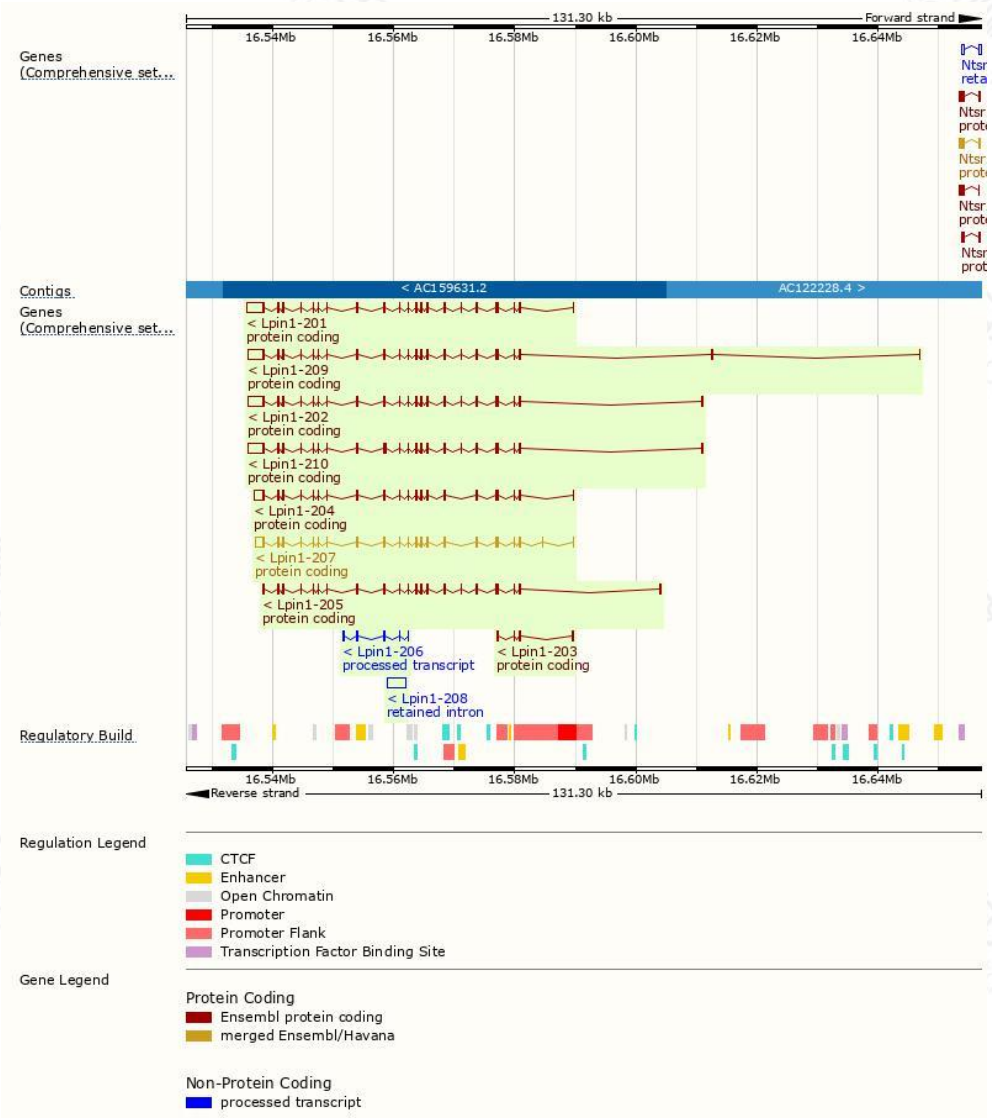
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lpin1-201	ENSMUST00000067124.5	5581	924aa	Protein coding	CCDS25822	E9QKQ5	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 P4
Lpin1-202	ENSMUST00000111067.9	5433	924aa	Protein coding	CCDS25822	E9QKQ5	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 P4
Lpin1-204	ENSMUST00000221230.1	4114	891aa	Protein coding	CCDS25823	A0A1Y7VLN4	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 ALT2
Lpin1-207	ENSMUST00000222989.1	4103	891aa	Protein coding	CCDS25823	A0A1Y7VLN4	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 ALT2
Lpin1-205	ENSMUST00000221297.1	3100	924aa	Protein coding	CCDS25822	E9QKQ5	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 P4
Lpin1-210	ENSMUST00000239165.1	5511	950aa	Protein coding	-	-	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
Lpin1-209	ENSMUST00000238839.1	5430	973aa	Protein coding	-	-	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
Lpin1-203	ENSMUST00000221146.1	707	158aa	Protein coding	-	A0A1Y7VJ01	CDS 3' incomplete TSL:3
Lpin1-206	ENSMUST00000221789.1	624	No protein	Processed transcript	-	-	TSL:3
Lpin1-208	ENSMUST00000223129.1	3022	No protein	Retained intron	-	-	TSL:NA

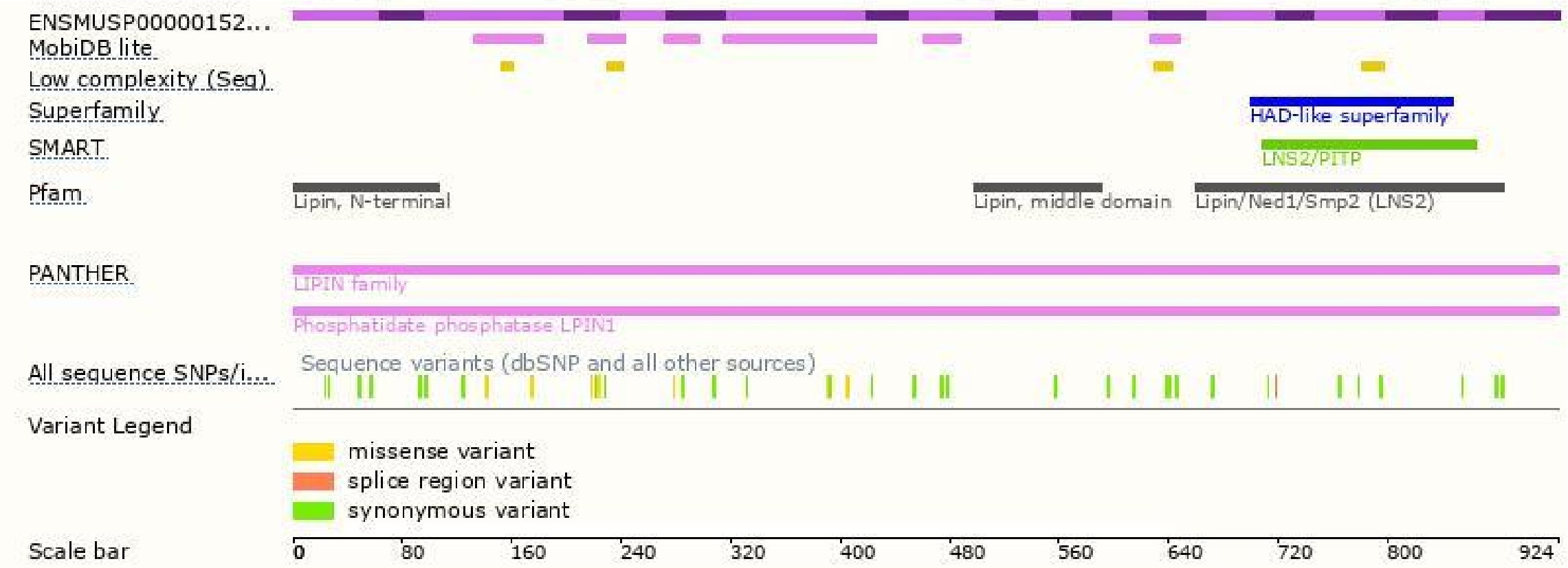
The strategy is based on the design of *Lpin1-201* transcript,the transcription is shown below:



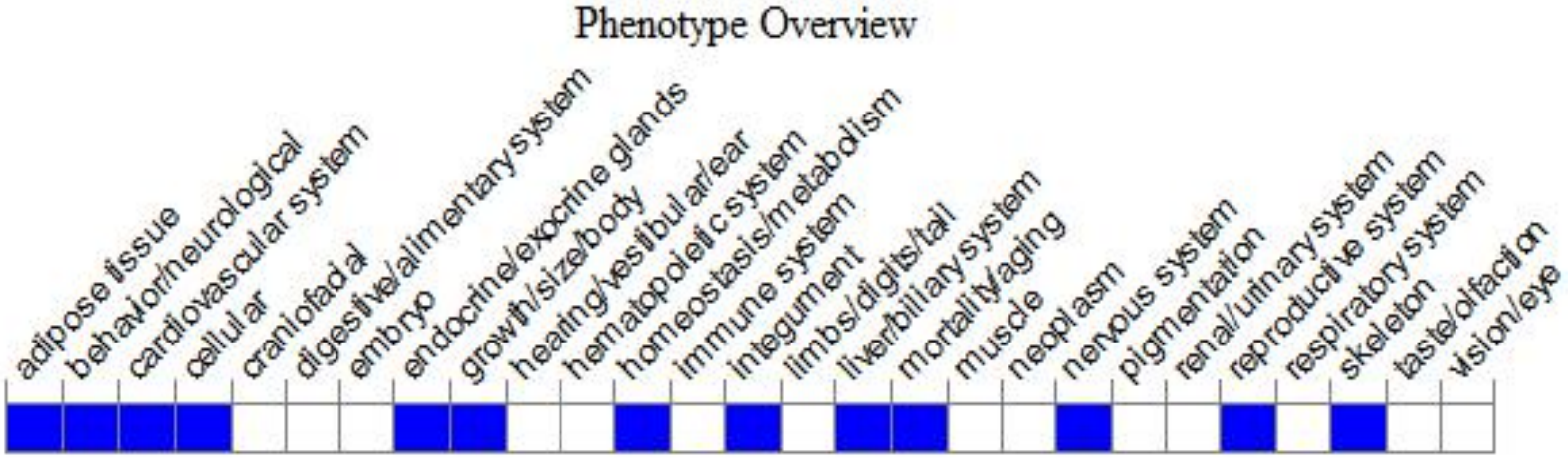
Genomic location distribution



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,eNU-induced mutants show transient hindlimb paralysis, demyelination and myelin sheath defects. Spontaneous mutants show neonatal fatty liver and hypertriglyceridemia, runting, male sterility, peripheral neuropathy, and altered hair growth, myelination, adipogenesis and lipid and glucose metabolism.

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

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