Hmgb1 Cas9-CKO Strategy

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Project Overview

Project Name	***Hmgb1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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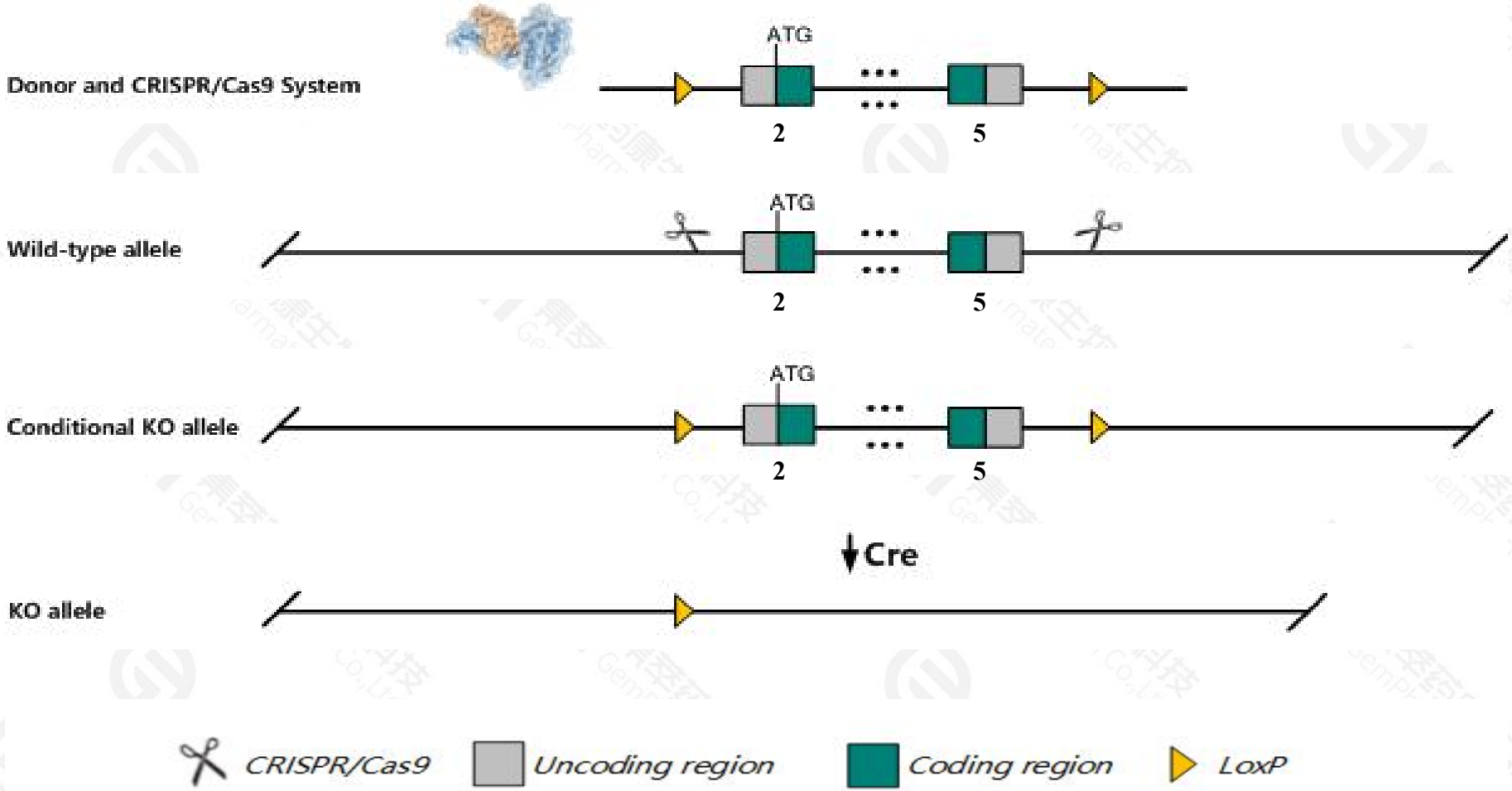
This model will use CRISPR/Cas9 technology to edit the *Hmgb1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Hmgb1* gene has 10 transcripts. According to the structure of *Hmgb1* gene, exon2-exon5 of *Hmgb1-201*(ENSMUST00000085546.13) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Hmgb1* 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,homozygous null mice display partial or complete neonatal lethality due to hypoglycemia depending on the strain background, with open eyelids at birth, atelectasis, and lethargy.

➢ The *Hmgb1* 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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Hmgb1 high mobility group box 1 [Mus musculus (house mouse)]

Gene ID: 15289, updated on 13-Mar-2020

Summary

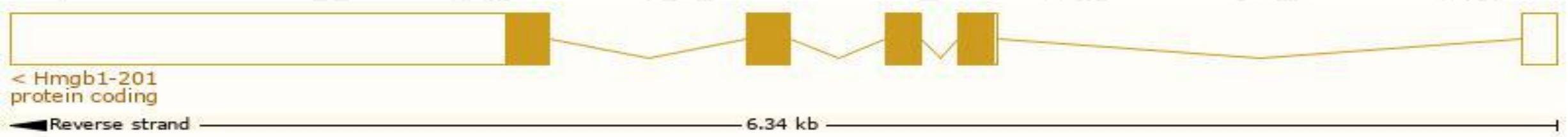
Official Symbol	Hmgb1 provided by MGI
Official Full Name	high mobility group box 1 provided byMGI
Primary source	MGI:MGI:96113
See related	Ensembl:ENSMUSG00000066551
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	HMG-1, Hmg1, SBP-1, p30
Summary	This gene encodes a protein that belongs to the High Mobility Group-box superfamily. The encoded non-histone, nuclear DNA-binding protein regulates transcription, and is involved in organization of DNA. This protein plays a role in several cellular processes, including inflammation, cell differentiation and tumor cell migration. Multiple pseudogenes of this gene have been identified. Alternative splicing results in multiple transcript variants that encode the same protein. [provided by RefSeq, Sep 2015]
Expression	Broad expression in CNS E11.5 (RPKM 22.0), liver E14 (RPKM 17.0) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

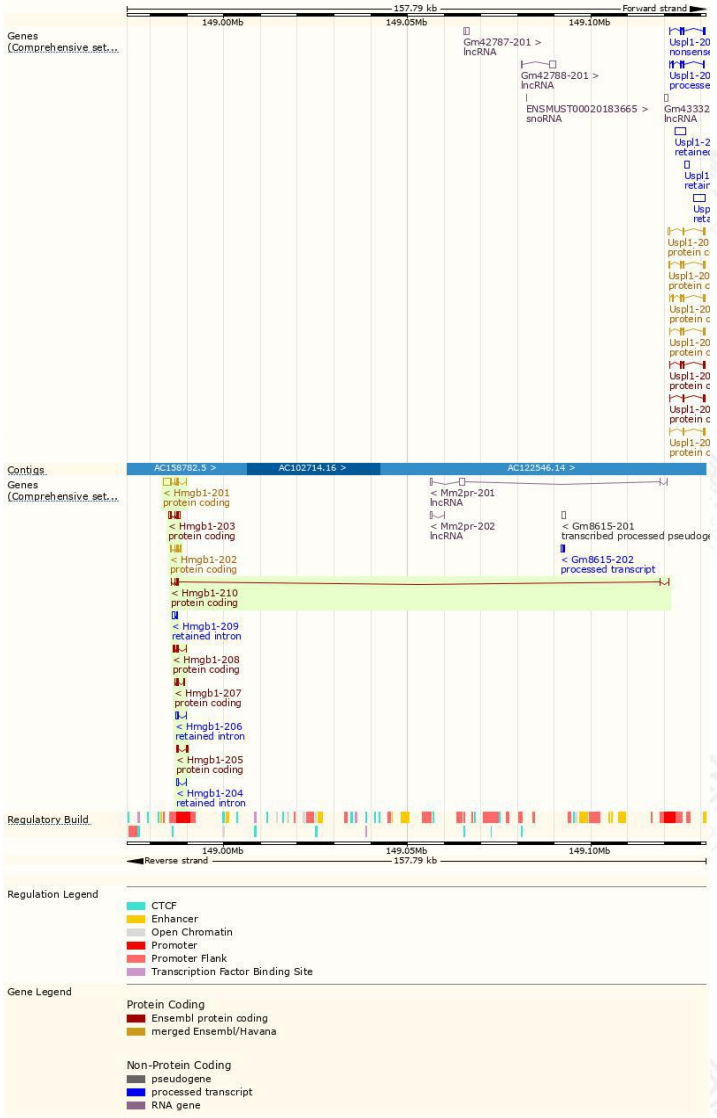
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hmgb1-201	ENSMUST00000085546.12	2838	215aa	Protein coding	CCDS19883	P63158 Q58EV5	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 P1
Hmgb1-203	ENSMUST00000110505.7	1720	215aa	Protein coding	CCDS19883	P63158 Q58EV5	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 P1
Hmgb1-202	ENSMUST00000093196.10	1092	215aa	Protein coding	CCDS19883	P63158 Q58EV5	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 P1
Hmgb1-208	ENSMUST00000139443.7	923	171aa	Protein coding	-	A0A0J9YUD8	TSL:2 GENCODE basic
Hmgb1-210	ENSMUST00000202133.3	685	211aa	Protein coding	-	A0A0J9YUZ4	CDS 3' incomplete TSL:3
Hmgb1-205	ENSMUST00000125605.1	604	92aa	Protein coding	-	D3YVC6	CDS 3' incomplete TSL:5
Hmgb1-207	ENSMUST00000138553.7	428	124aa	Protein coding	-	D3YZ18	CDS 3' incomplete TSL:2
Hmgb1-209	ENSMUST00000155086.1	902	No protein	Retained intron	-	-	TSL:2
Hmgb1-206	ENSMUST00000133667.1	654	No protein	Retained intron	-	-	TSL:2
Hmgb1-204	ENSMUST00000123645.1	461	No protein	Retained intron	-	-	TSL:2

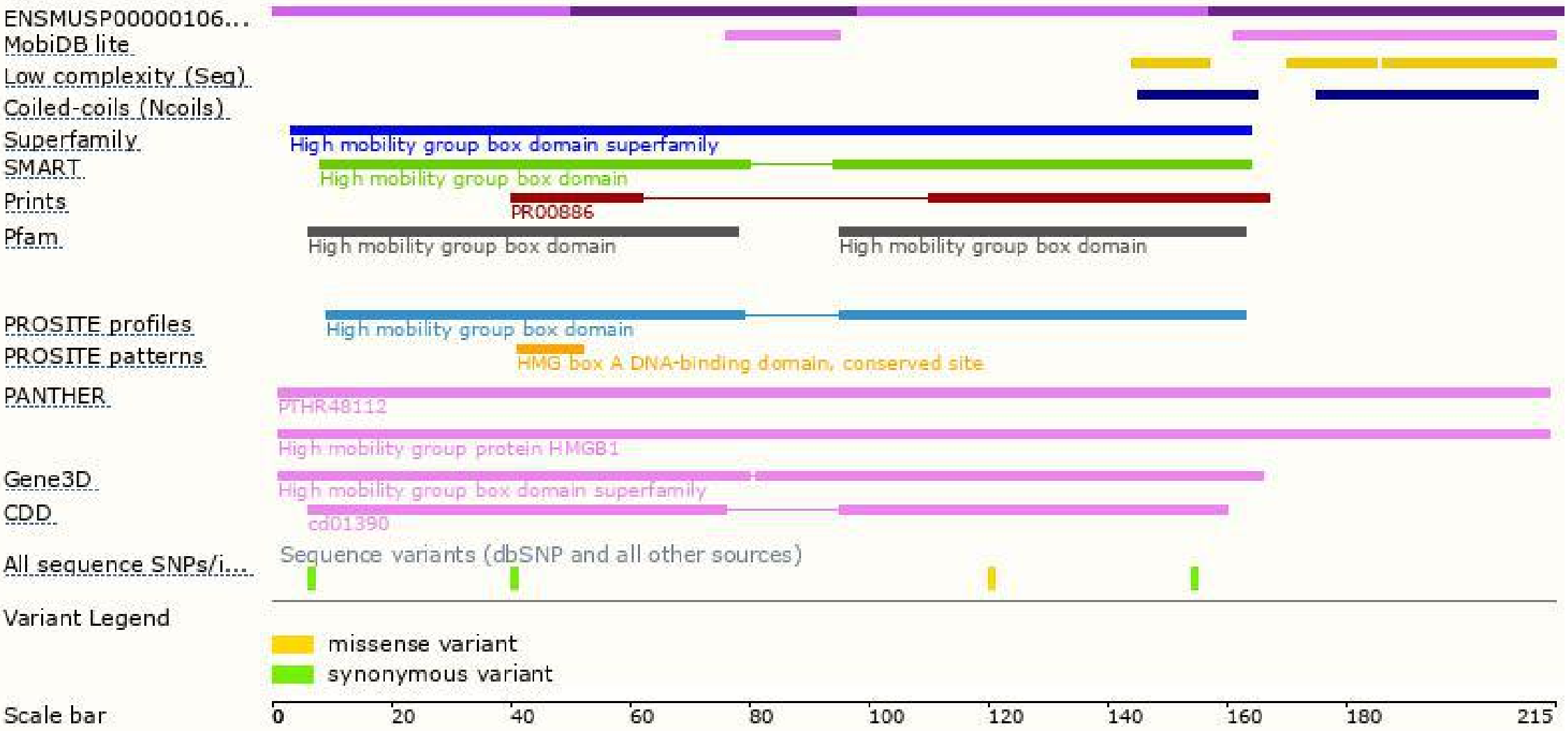
The strategy is based on the design of *Hmgb1-201* transcript,the transcription is shown below:



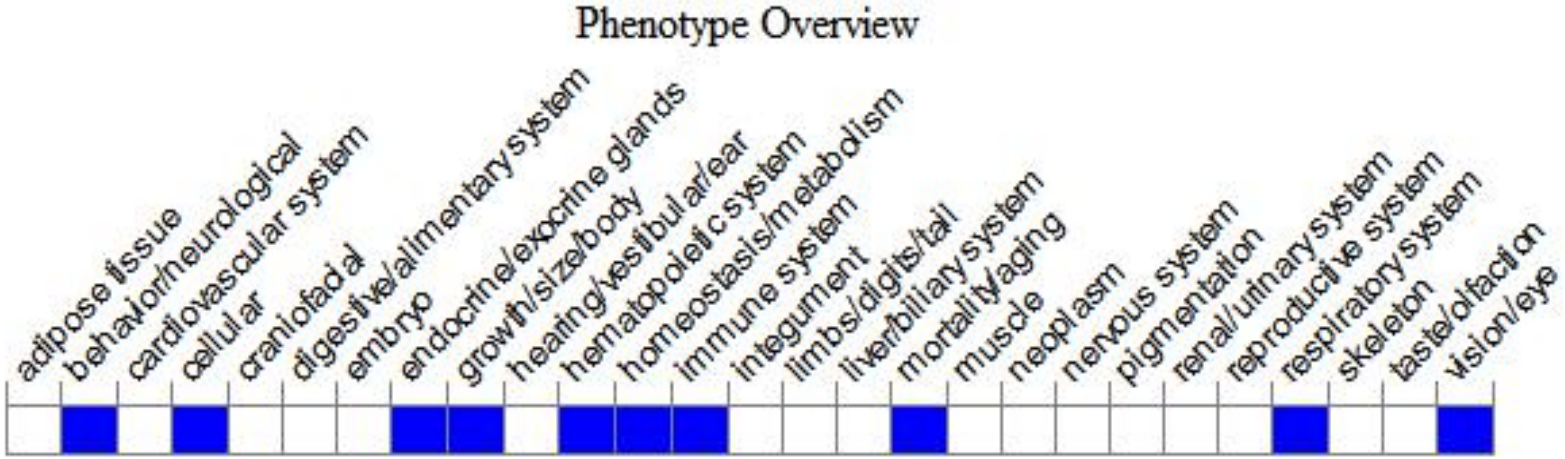
Genomic location distribution



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,homozygous null mice display partial or complete neonatal lethality due to hypoglycemia depending on the strain background, with open eyelids at birth, atelectasis, and lethargy.

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