Pde8b Cas9-CKO Strategy

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Reviewer: Yanhua Shen

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Project Overview

Project Name	***Pde8b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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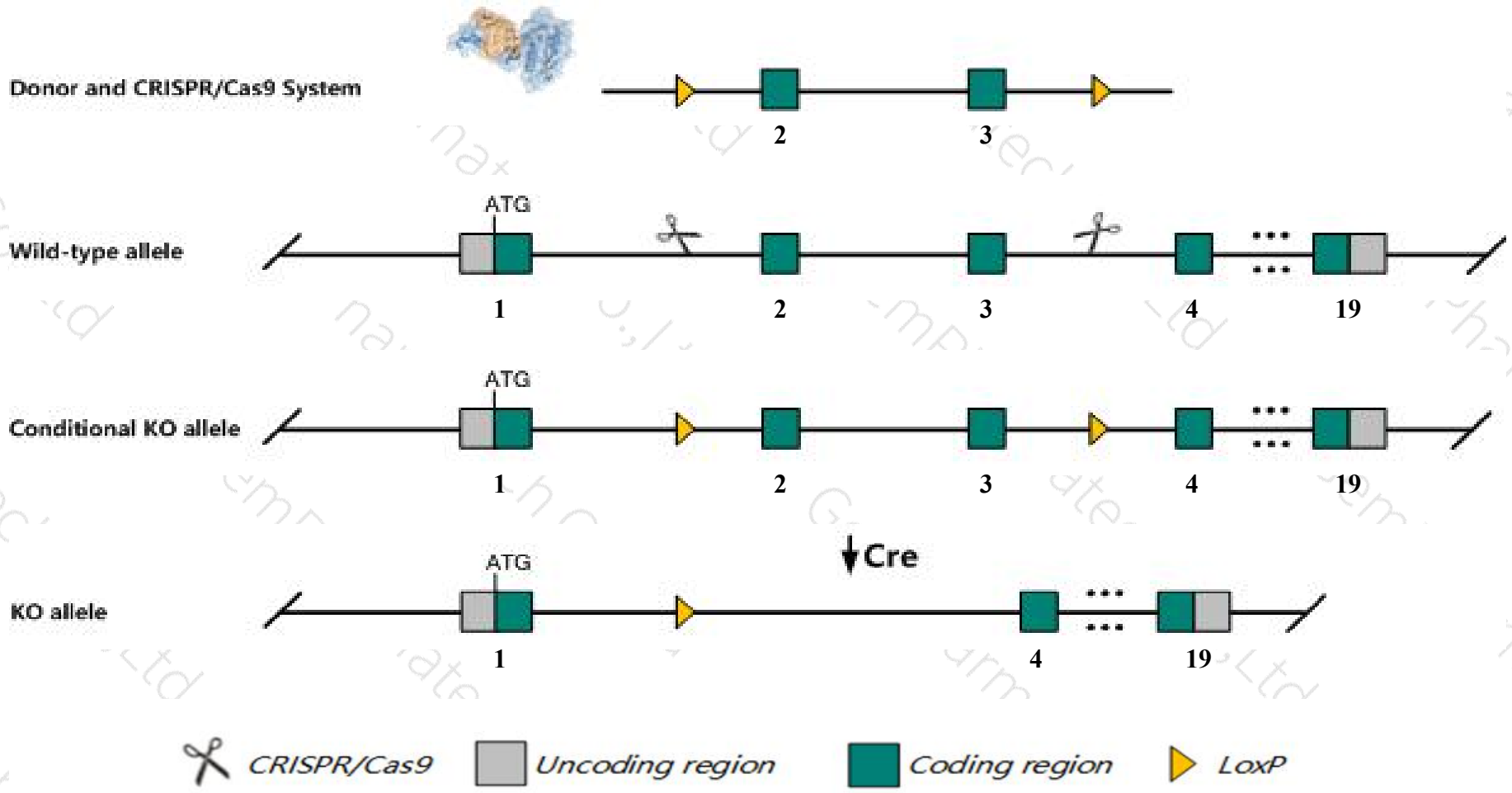
This model will use CRISPR/Cas9 technology to edit the *Pde8b* gene. The schematic diagram is as follows:



Technical routes

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- The *Pde8b* gene has 14 transcripts. According to the structure of *Pde8b* gene, exon2-exon3 of *Pde8b-208* (ENSMUST00000162292.7) transcript is recommended as the knockout region.The region contains 251bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Pde8b* 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 exhibit increased uring corticosterone, decreased serum adrenocorticotropin and decreased sensitivity to a pde8-selective inhibitor.
- Transcript *Pde8b*-205&213 will be destroyed.
- The effect on transcript *Pde8b*-211 is unknown.
- The *Pde8b* gene is located on the Chr13. 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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Pde8b phosphodiesterase 8B [Mus musculus (house mouse)]

Gene ID: 218461, updated on 13-Mar-2020

Summary

Official Symbol	Pde8b provided by MGI
Official Full Name	phosphodiesterase 8B provided byMGI
Primary source	MGI:MGI:2443999
See related	Ensembl:ENSMUSG00000021684
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	B230331L10Rik, C030047E14Rik
Expression	Biased expression in adrenal adult (RPKM 17.9), cortex adult (RPKM 8.4) and 9 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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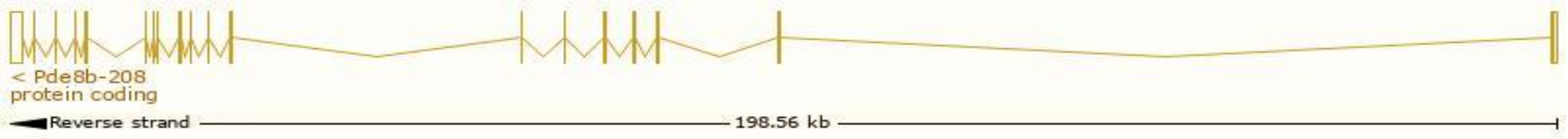
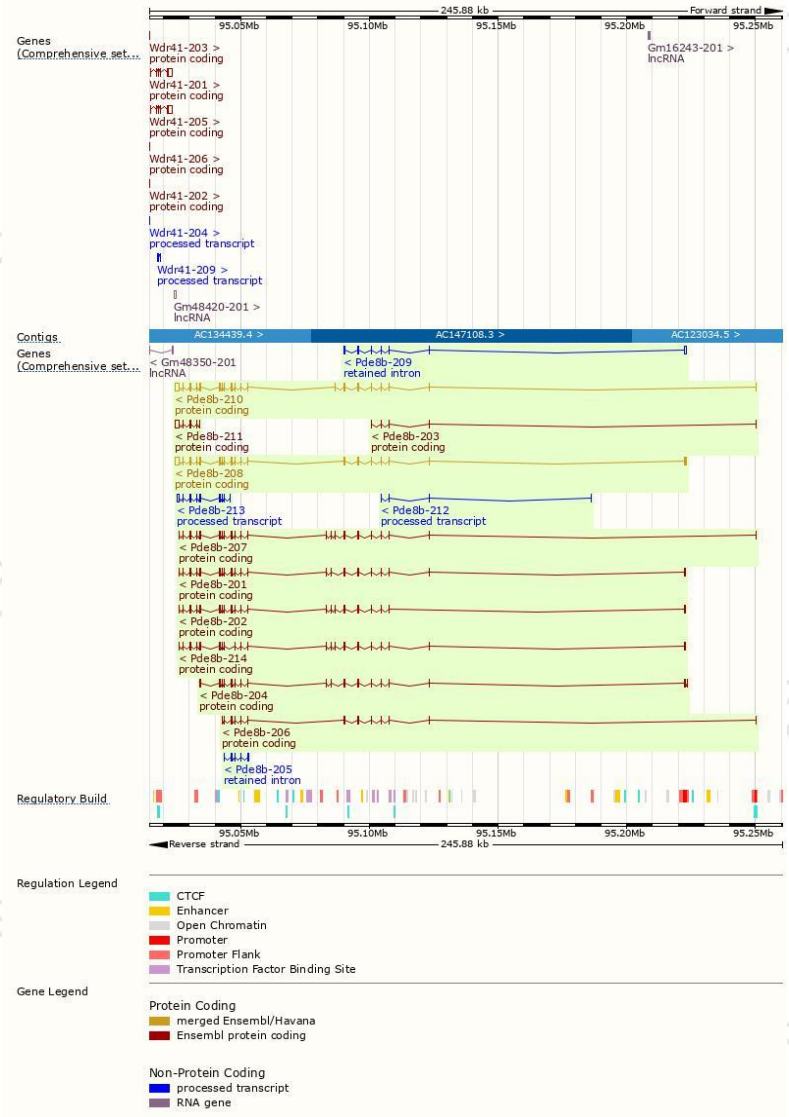
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pde8b-208	ENSMUST00000162292.7	4235	788aa	Protein coding	CCDS49331	E9PYP0	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 P3
Pde8b-210	ENSMUST00000162412.7	4035	730aa	Protein coding	CCDS49332	E9PXX4	TSL:1 GENCODE basic
Pde8b-207	ENSMUST00000162153.8	2474	780aa	Protein coding	CCDS84052	E9PX38	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
Pde8b-202	ENSMUST00000067082.13	2598	865aa	Protein coding	-	E9Q4S1	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
Pde8b-201	ENSMUST00000022192.11	2517	838aa	Protein coding	-	E9Q8J6	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
Pde8b-214	ENSMUST00000172104.7	2493	830aa	Protein coding	-	E9Q7S6	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
Pde8b-204	ENSMUST00000159608.7	2370	674aa	Protein coding	-	E9PUL4	TSL:5 GENCODE basic
Pde8b-211	ENSMUST00000162670.7	2363	196aa	Protein coding	-	F7CT85	CDS 5' incomplete TSL:1
Pde8b-206	ENSMUST00000160957.8	1101	329aa	Protein coding	-	E0CXQ1	CDS 3' incomplete TSL:5
Pde8b-203	ENSMUST00000159598.1	402	102aa	Protein coding	-	E0CY70	CDS 3' incomplete TSL:5
Pde8b-213	ENSMUST00000162882.1	2060	No protein	Processed transcript	-	-	TSL:1
Pde8b-212	ENSMUST00000162716.1	539	No protein	Processed transcript	-	-	TSL:3
Pde8b-209	ENSMUST00000162328.7	1338	No protein	Retained intron	-	-	TSL:1
Pde8b-205	ENSMUST00000160412.1	456	No protein	Retained intron	-	-	TSL:5

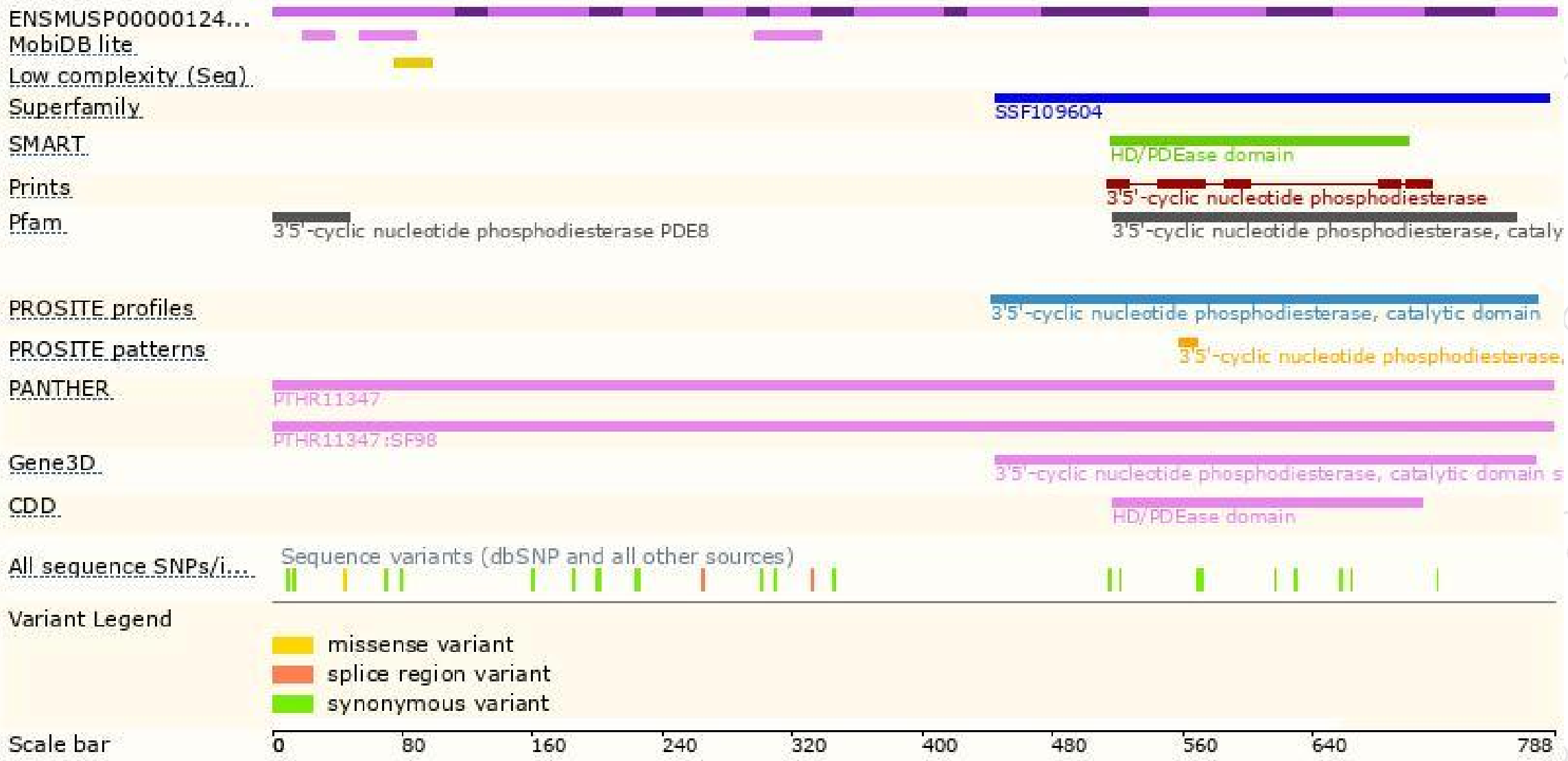
The strategy is based on the design of *Pde8b-208* transcript,the transcription is shown below:



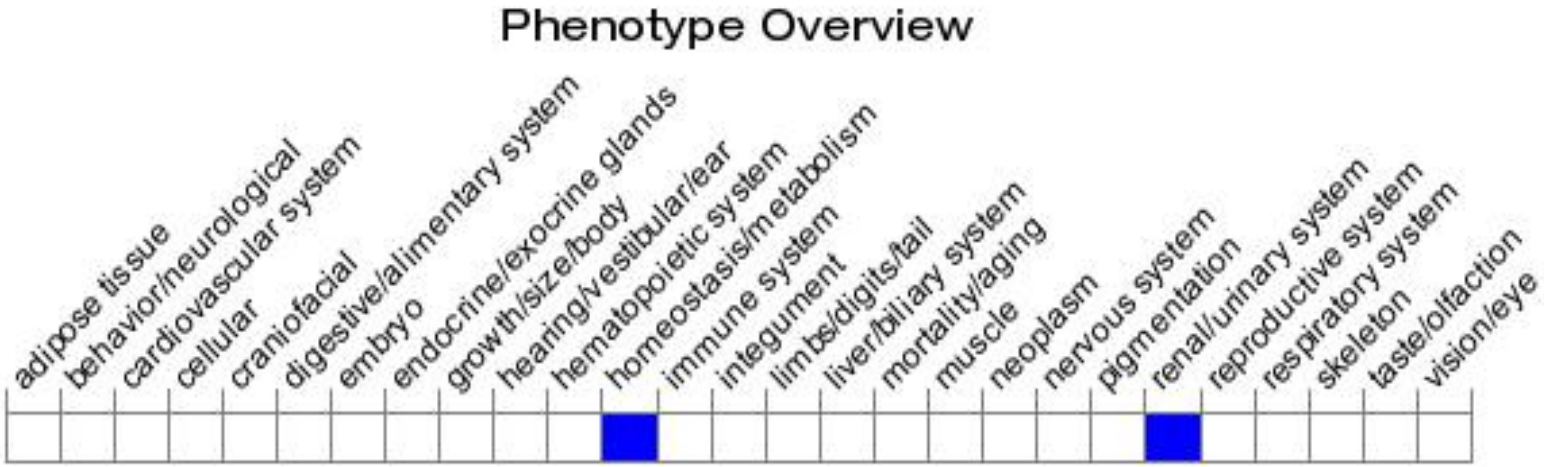
Genomic location distribution



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 exhibit increased uring corticosterone, decreased serum adrenocorticotropin and decreased sensitivity to a PDE8-selective inhibitor.

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