Arntl Cas9-CKO Strategy

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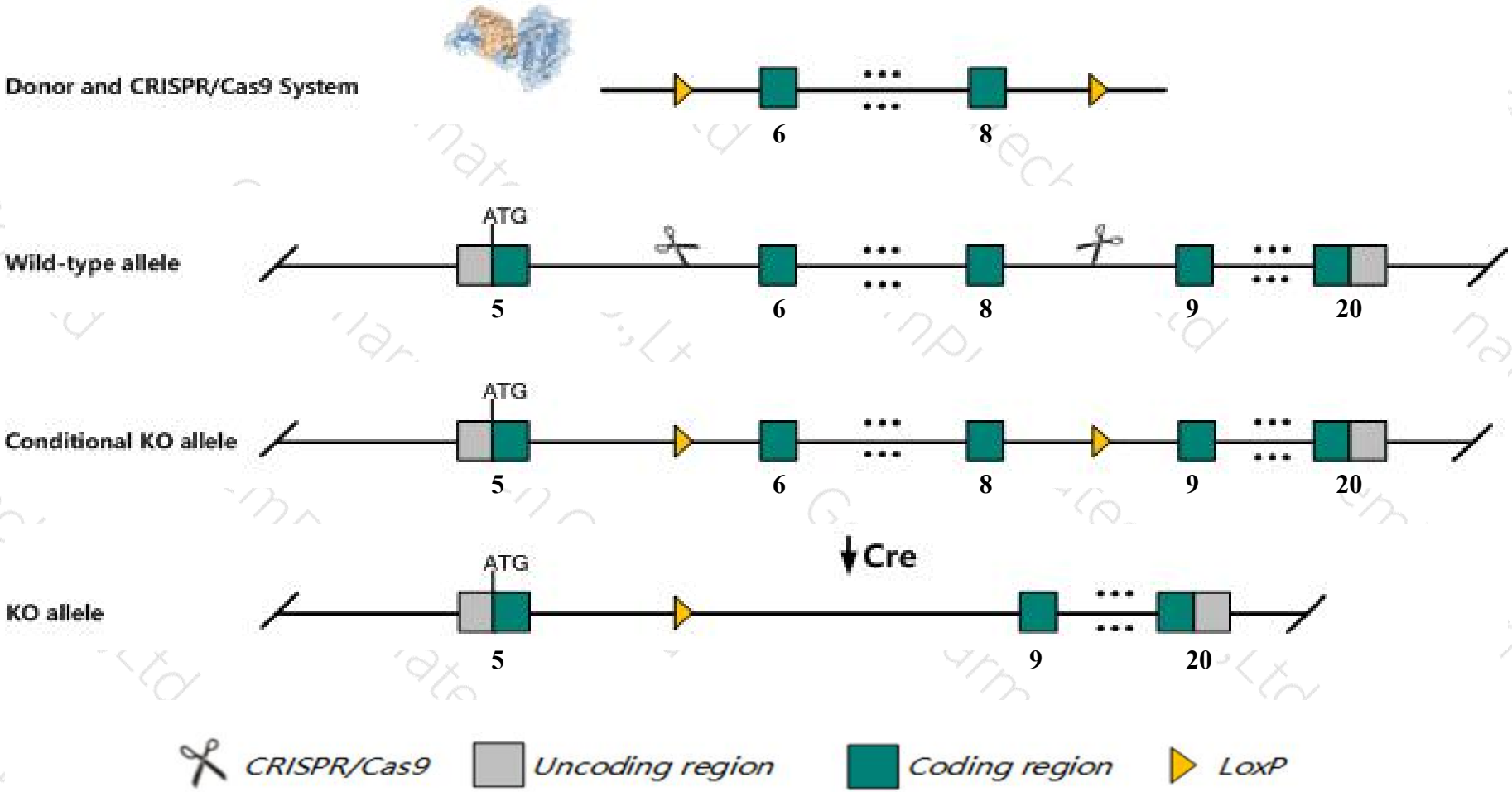
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Arntl* gene has 6 transcripts. According to the structure of *Arntl* gene, exon6-exon8 of *Arntl-201* (ENSMUST00000047321.8) transcript is recommended as the knockout region.The region contains 239bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Arntl* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 mutation of this gene results in abnormal light/dark cycle activity and decreases overall activity levels. Mice homozygous for another knock-out allele exhibit loss of circadian rhythm in locomotor activity, dyslipidemia, ectopic fat formationand altered energy homeostasis.

- The *Arntl* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Arntl aryl hydrocarbon receptor nuclear translocator-like [Mus musculus (house mouse)]

Gene ID: 11865, updated on 9-Apr-2019

Summary

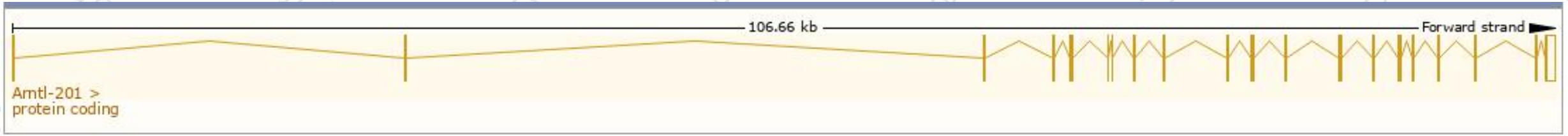
Official Symbol	Arntl provided by MGI
Official Full Name	aryl hydrocarbon receptor nuclear translocator-like provided by MGI
Primary source	MGI:MGI:1096381
See related	Ensembl:ENSMUSG00000055116
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Arnt3, BMAL1b, Bmal1, MOP3, bHLHe5, bmal1b'
Summary	The protein encoded by this gene is a basic helix-loop-helix protein that forms a heterodimer with Clock. This heterodimer binds E-box enhancer elements upstream of Period (Per1, Per2, Per3) and Cryptochrome (Cry1, Cry2) genes and activates transcription of these genes. Per and Cry proteins heterodimerize and repress their own transcription by interacting in a feedback loop with Clock/Arntl complexes. Defects in this gene have been linked to infertility, problems with gluconeogenesis and lipogenesis, and altered sleep patterns. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jan 2014]
Expression	Ubiquitous expression in thymus adult (RPKM 4.9), large intestine adult (RPKM 4.6) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

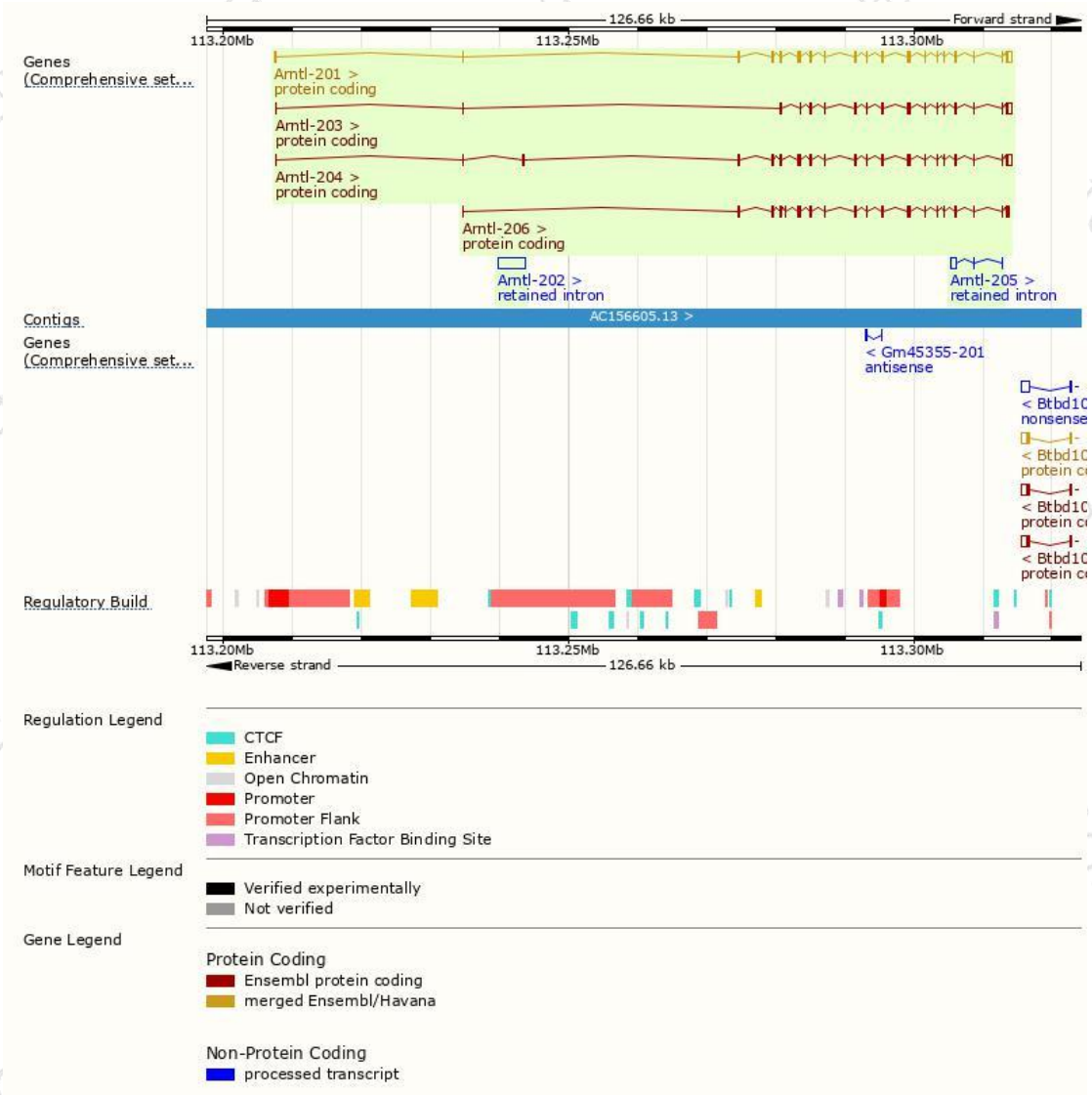
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arntl-204	ENSMUST00000210238.1	2976	626aa	Protein coding	CCDS40092	Q3UHZ2 Q9WTL8	TSL:5 GENCODE basic APPRIS P1
Arntl-201	ENSMUST00000047321.8	2908	626aa	Protein coding	CCDS40092	Q3UHZ2 Q9WTL8	TSL:1 GENCODE basic APPRIS P1
Arntl-203	ENSMUST00000210074.1	2554	613aa	Protein coding	CCDS85390	Q9WTL8	TSL:1 GENCODE basic
Arntl-206	ENSMUST00000211770.1	2464	633aa	Protein coding	-	A0A1B0GS77	TSL:5 GENCODE basic
Arntl-202	ENSMUST00000209495.1	3995	No protein	Retained intron	-	-	TSL:NA
Arntl-205	ENSMUST00000211547.1	846	No protein	Retained intron	-	-	TSL:5

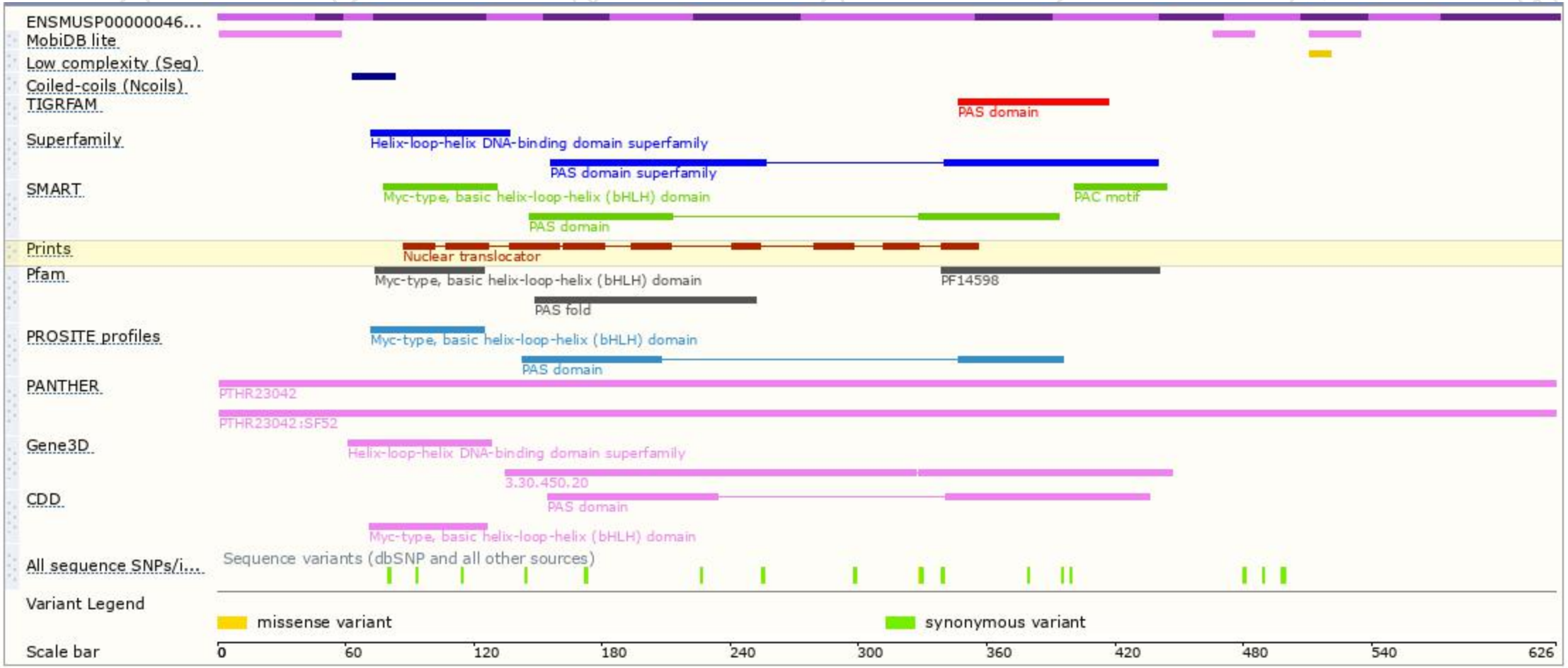
The strategy is based on the design of *Arntl-201* transcript,The transcription is shown below



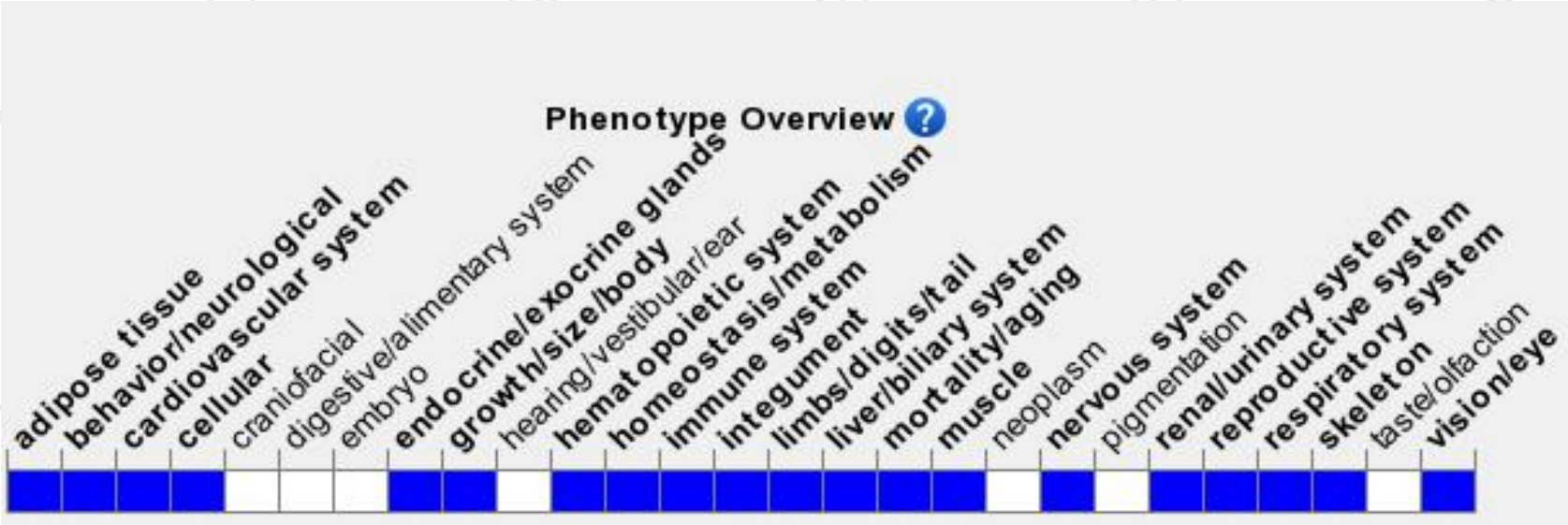
Genomic location distribution



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 mutation of this gene results in abnormal light/dark cycle activity and decreases overall activity levels. Mice homozygous for another knock-out allele exhibit loss of circadian rhythm in locomotor activity, dyslipidemia, ectopic fat formationand altered energy homeostasis.

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

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