

Agrn Cas9-CKO Strategy

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Reviewer:

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Design Date:

2019/9/11

Project Overview

Project Name

Agrn

Project type

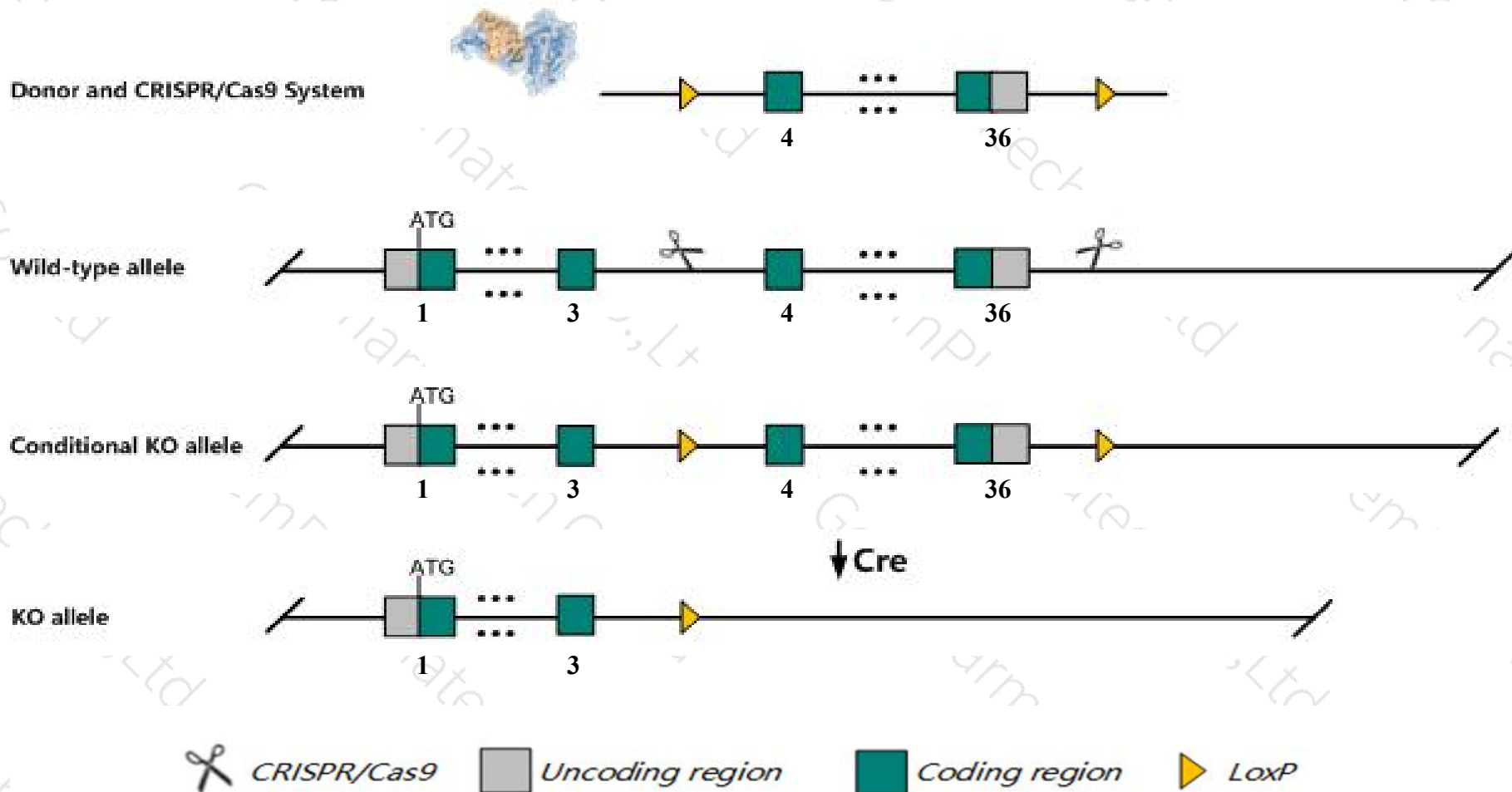
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Agrn* gene has 7 transcripts. According to the structure of *Agrn* gene, exon4-exon36 of *Agrn*-206 (ENSMUST00000180572.1) transcript is recommended as the knockout region. The region contains most of coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Agrn* 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.

- According to the existing MGI data, Nullizygous mice display embryonic failure of NMJ formation, inability to breathe or move and perinatal lethality. Homozygotes for an ENU-induced allele show poor hindlimb motor control, myopathy, muscle atrophy, spasms and fiber-type switching, NMJ disaggregation, camptodactyly and premature death.
- The *Agrn* gene is located on the Chr4. 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)

Agrn agrin [*Mus musculus* (house mouse)]

Gene ID: 11603, updated on 3-Sep-2019

Summary

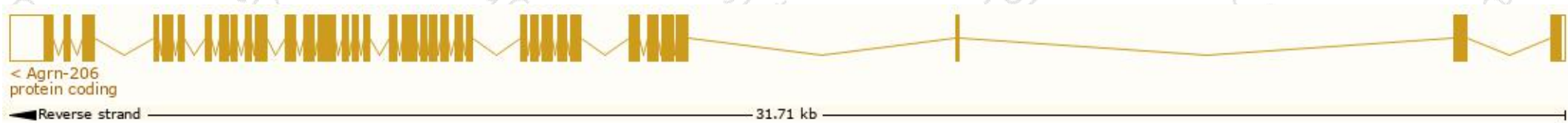
Official Symbol	Agrn provided by MGI
Official Full Name	agrin provided by MGI
Primary source	MGI:MGI:87961
See related	Ensembl:ENSMUSG000000041936
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Agrin; nmf380
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 86.1), CNS E14 (RPKM 82.7) and 27 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

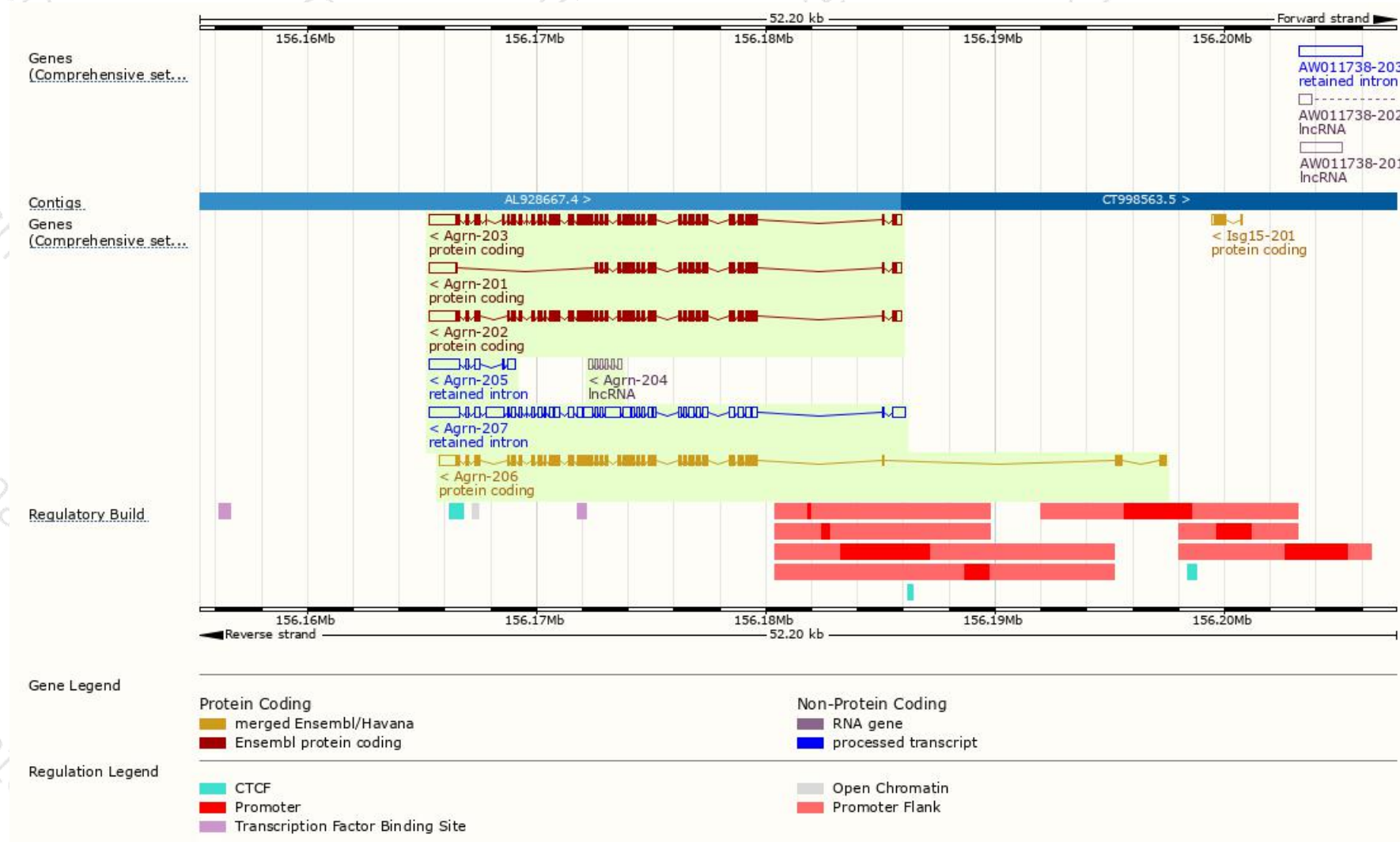
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Agrn-206	ENSMUST00000180572.1	6891	2034aa	Protein coding	CCDS19060	M0QWP1	TSL:5 GENCODE basic APPRIS P1
Agrn-203	ENSMUST00000105575.8	7262	1950aa	Protein coding	-	A2ASQ1	TSL:5 GENCODE basic
Agrn-202	ENSMUST00000105574.8	7193	1927aa	Protein coding	-	Z4YK85	TSL:5 GENCODE basic
Agrn-201	ENSMUST00000071248.11	4831	1151aa	Protein coding	-	Z4YJS5	TSL:5 GENCODE basic
Agrn-207	ENSMUST00000181062.7	8612	No protein	Retained intron	-	-	TSL:1
Agrn-205	ENSMUST00000154494.7	2071	No protein	Retained intron	-	-	TSL:1
Agrn-204	ENSMUST00000144749.1	762	No protein	lncRNA	-	-	TSL:2

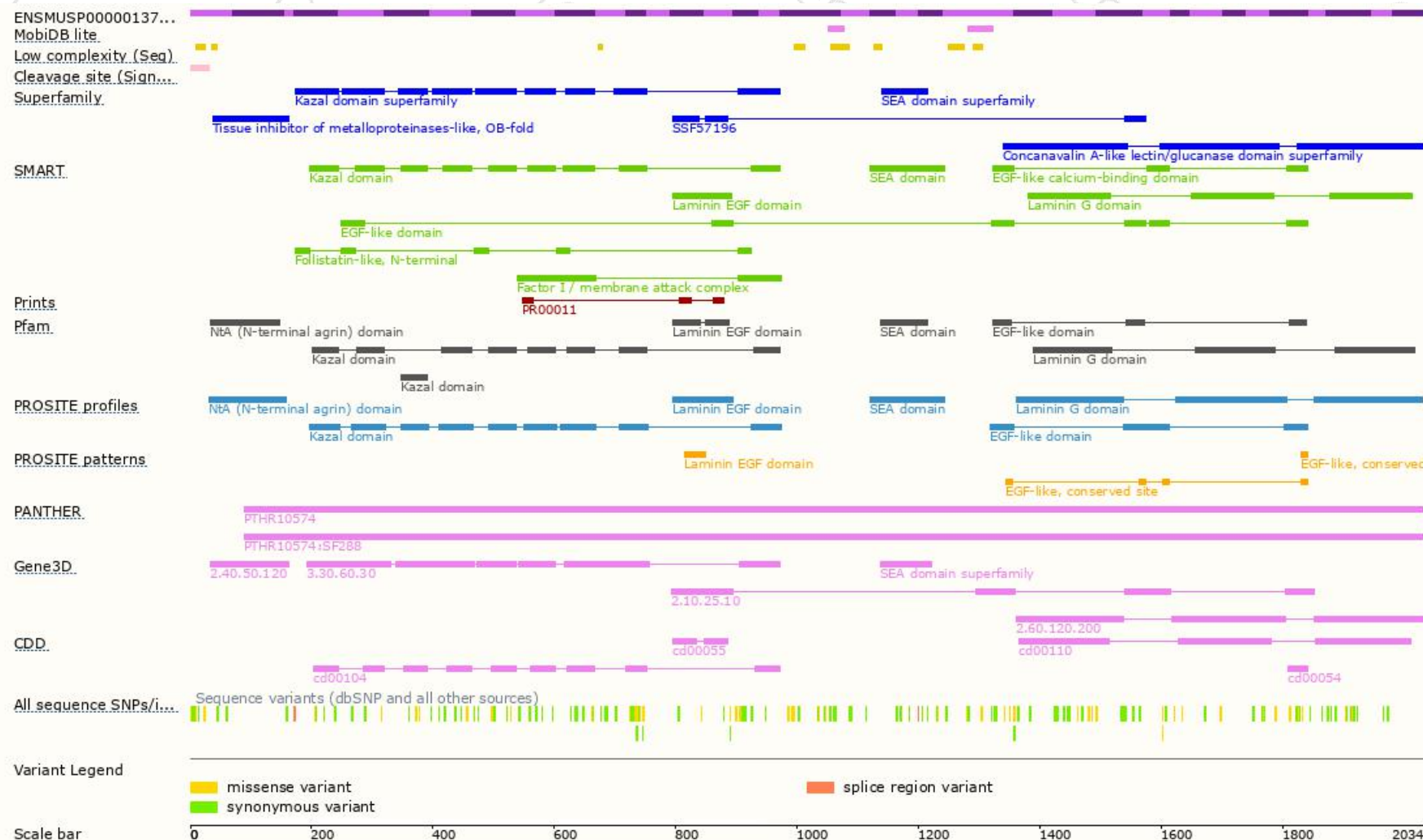
The strategy is based on the design of *Agrn-206* transcript,The transcription is shown below



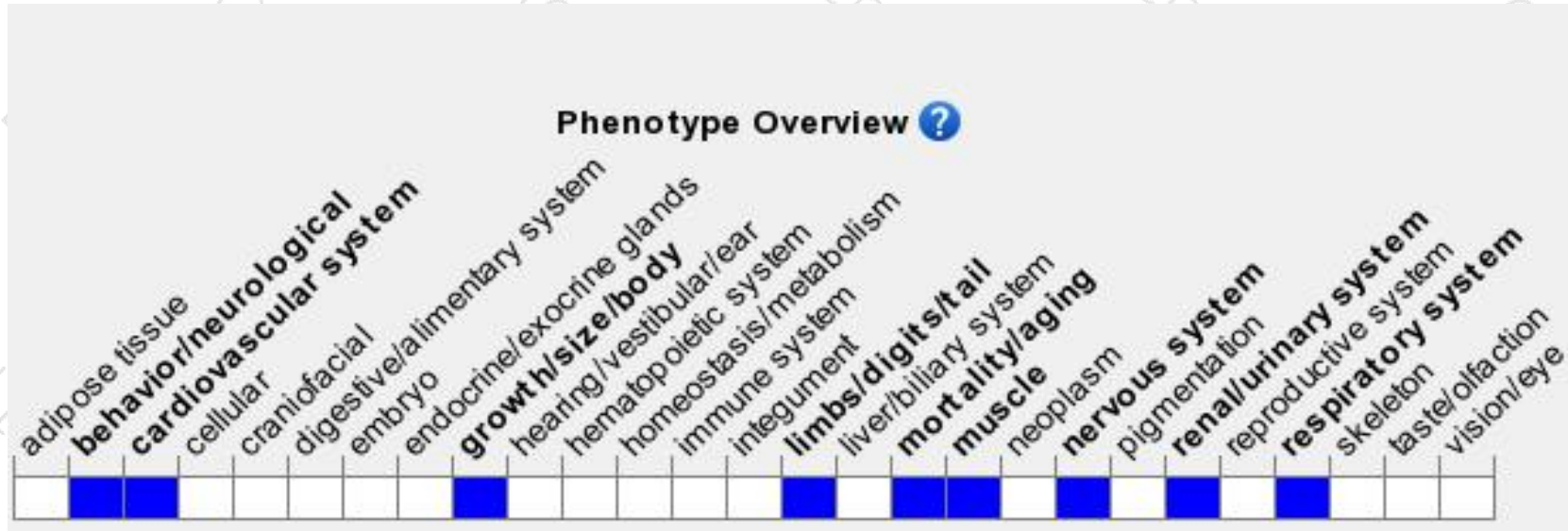
Genomic location distribution



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/>).

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

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