Arhgap35 Cas9-CKO Strategy

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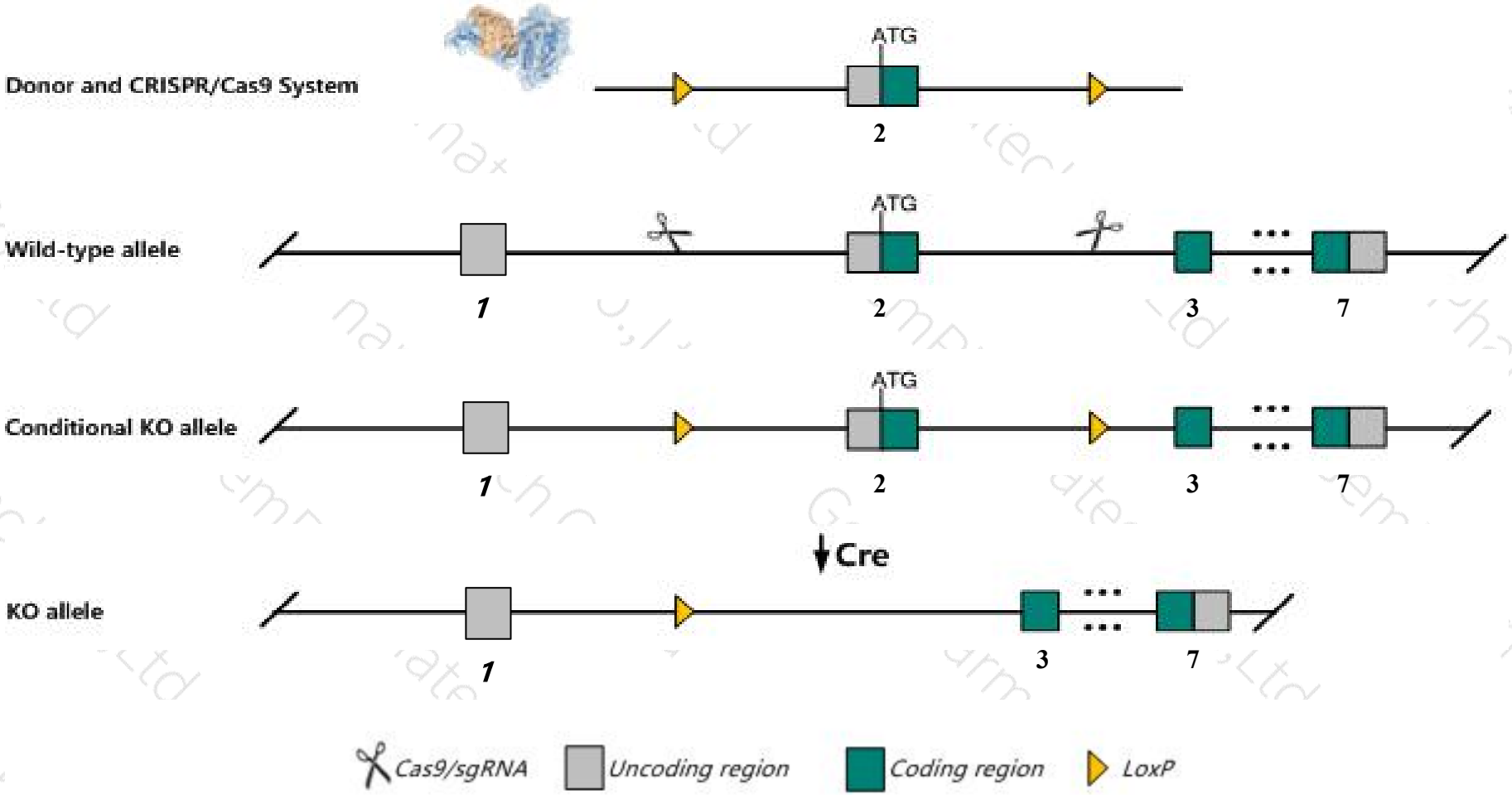
Design Date: 2020-7-23

Project Overview

Project Name	***Arhgap35***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Arhgap35* gene. The schematic diagram is as follows:



Technical routes

➢ The *Arhgap35* gene has 2 transcripts. According to the structure of *Arhgap35* gene, exon2 of *Arhgap35-201*(ENSMUST00000075845.10) transcript is recommended as the knockout region.The region contains start codon ATG.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Arhgap35* 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,mice homozygous for disruptions in this gene usually die within 2 days of birth and never survive beyond 3 weeks. Observed phenotypes include defects in eye morphogenesis, forebrain development, neural tube closure, axon guidance and fasciculation, and renal abnormalities, including hypoplastic and glomerulocystic kidneys, associated with a ciliogenesis defect.

➢ The *Arhgap35* 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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Arhgap35 Rho GTPase activating protein 35 [*Mus musculus* (house mouse)]

Gene ID: 232906, updated on 26-Jun-2020

Summary

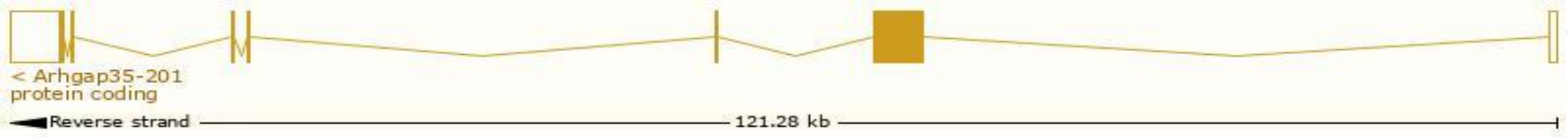
Official Symbol	Arhgap35 provided by MGI
Official Full Name	Rho GTPase activating protein 35 provided by MGI
Primary source	MGI:MGI:1929494
See related	Ensembl:ENSMUSG00000058230
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Grlf1; p190A; AI841135; mKIAA1722; p190RhoGAP; 6430596G11Rik
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 20.1), ovary adult (RPKM 18.9) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

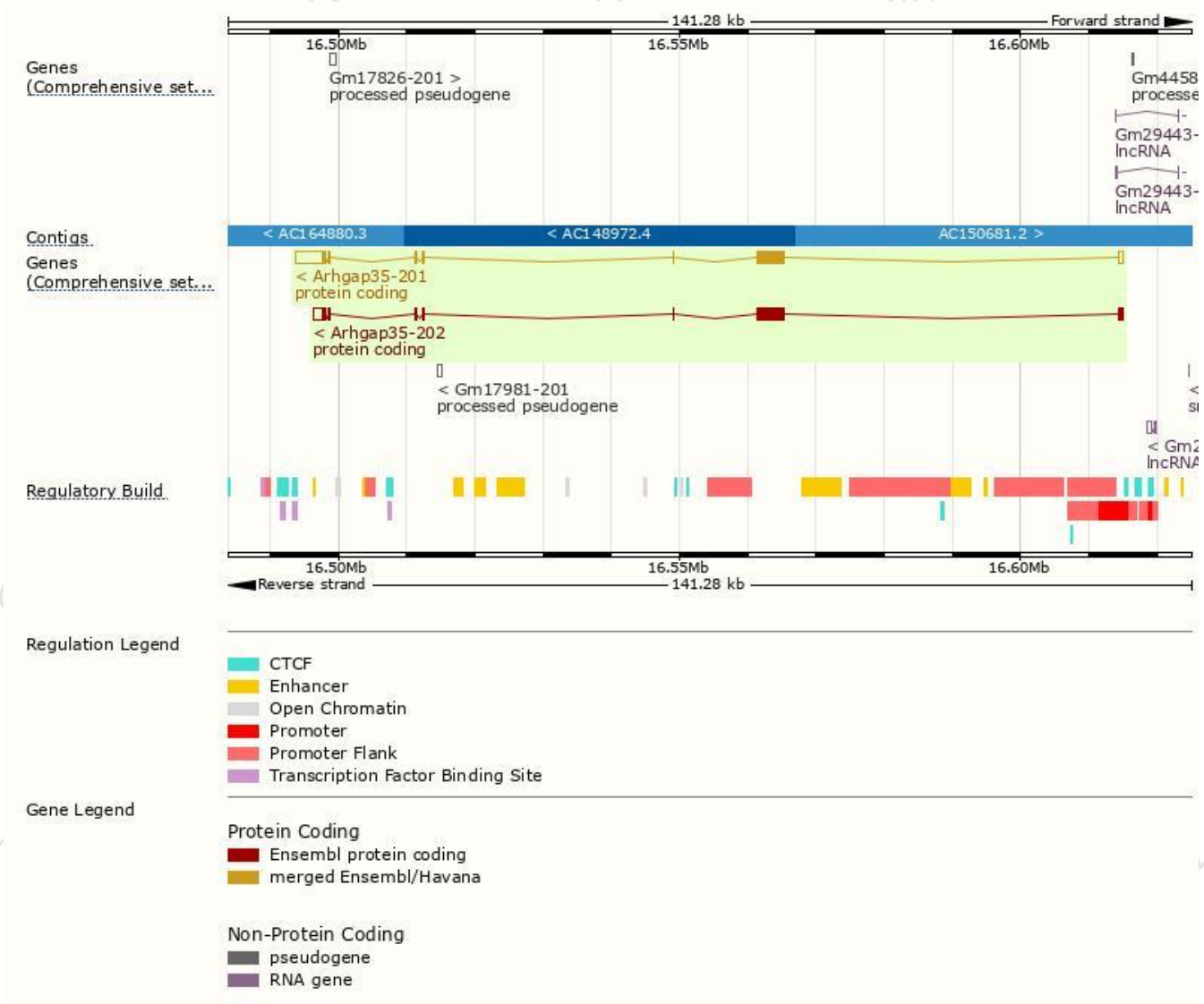
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arhgap35-201	ENSMUST00000075845.10	9147	1499aa	Protein coding	CCDS20851	B2RTN5 Q91YM2	TSL:1 GENCODE basic APPRIS P1
Arhgap35-202	ENSMUST00000171937.1	6251	1499aa	Protein coding	CCDS20851	B2RTN5 Q91YM2	TSL:5 GENCODE basic APPRIS P1

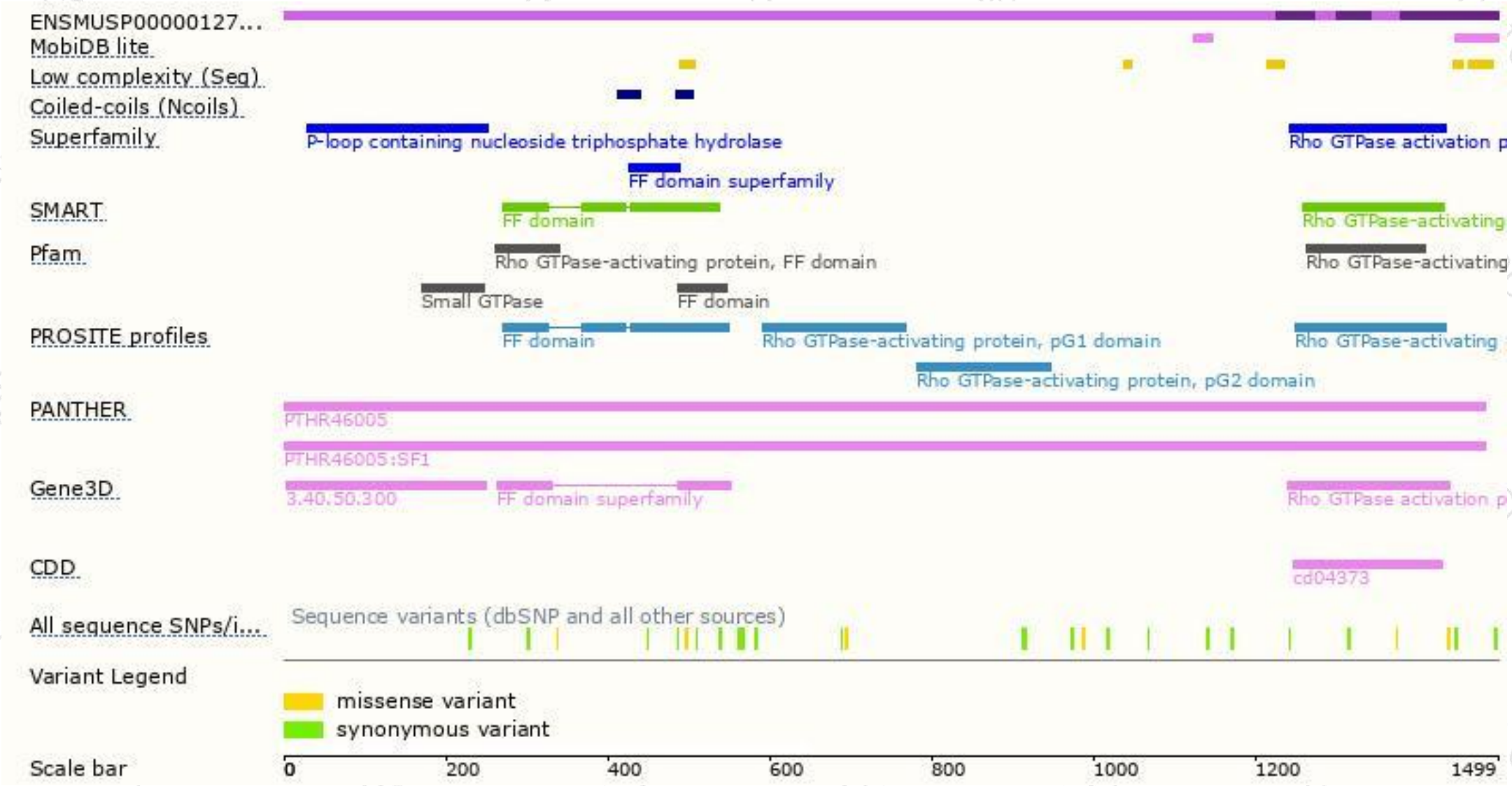
The strategy is based on the design of *Arhgap35-201* transcript,the transcription is shown below:



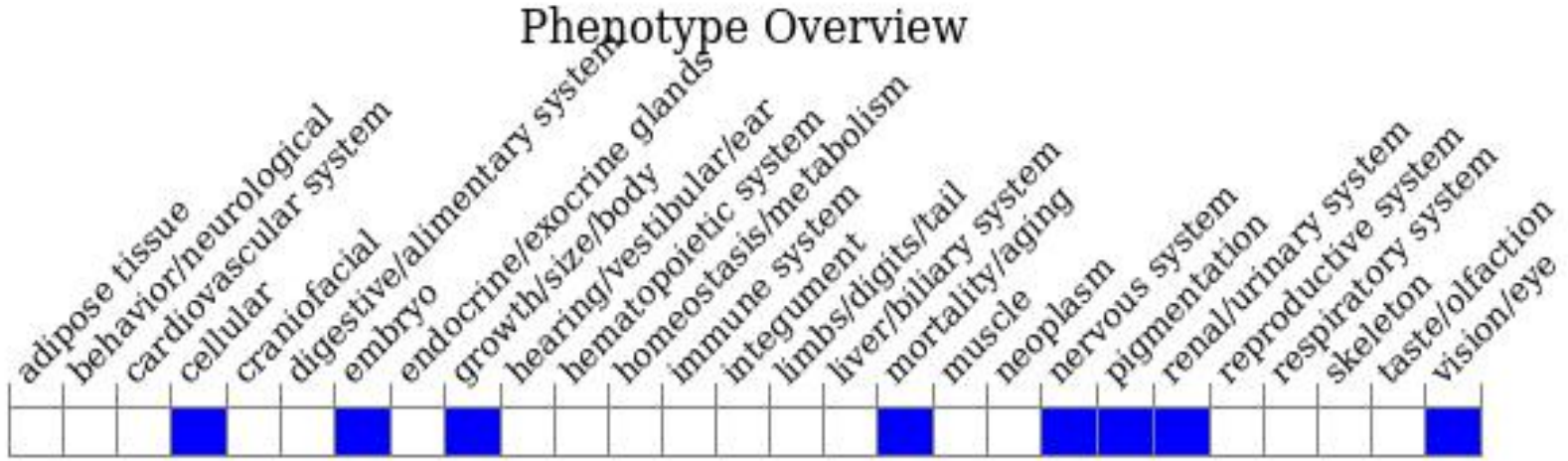
Genomic location distribution



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/).

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

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