

Arhgap5 Cas9-CKO Strategy

Designer: Xueting Zhang
Reviewer: Yanhua Shen
Design Date: 2019-8-28

Project Overview

Project Name

Arhgap5

Project type

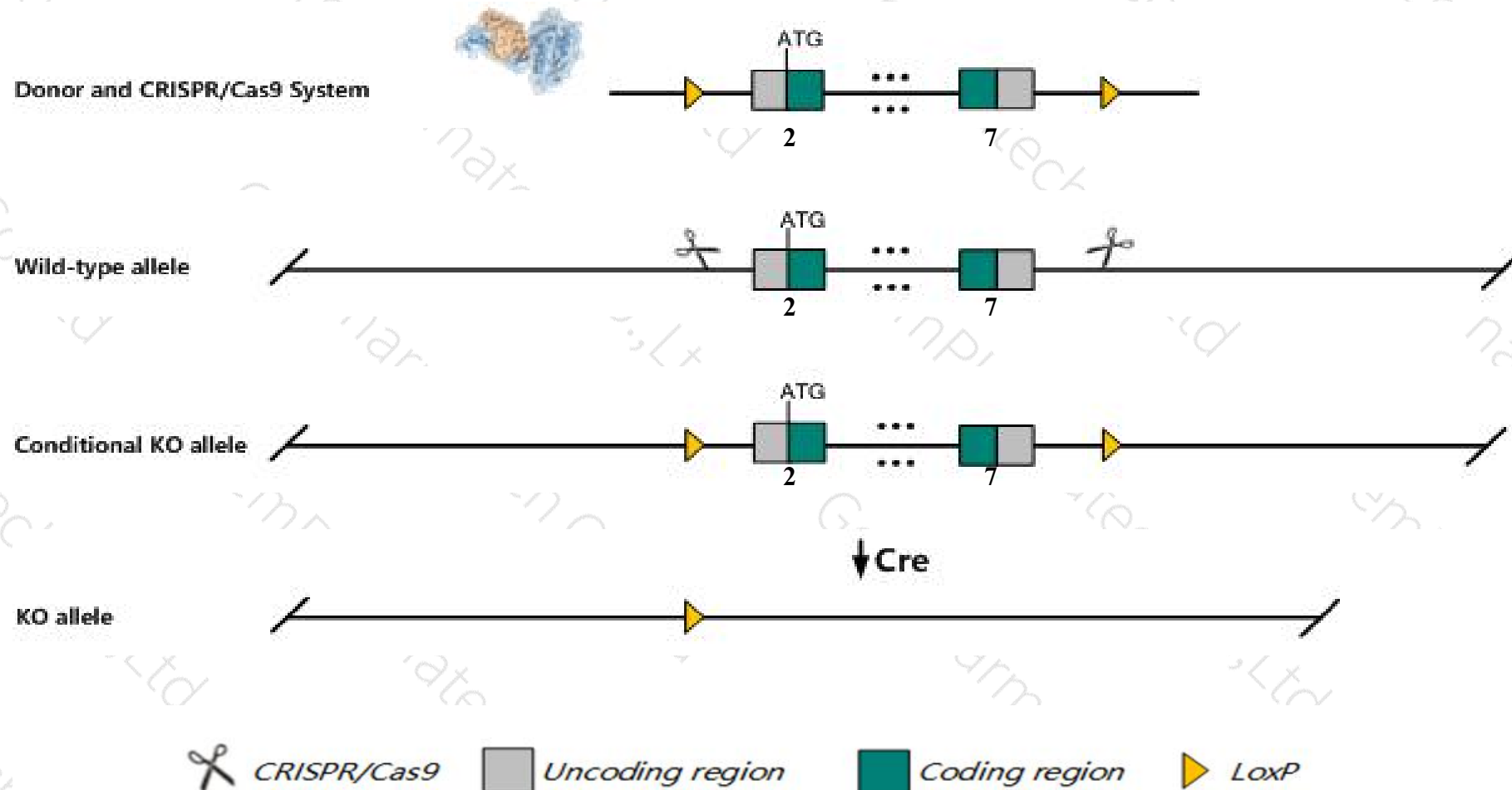
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Arhgap5* gene. The schematic diagram is as follows:



Technical routes

- The *Arhgap5* gene has 4 transcripts. According to the structure of *Arhgap5* gene, exon2-exon7 of *Arhgap5*-204 (ENSMUST00000219443.1) 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 *Arhgap5* 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, Homozygotes die at birth, are 30% smaller, do not inflate their lungs, and show a small thymus, abnormal adipocyte differentiation and brain defects in the corpus callosum, anterior commissure and lateral ventricles. Mutant MEFs show impaired adipogenesis but undergo myogenesis in response to IGF-1.
- *Arhgap5* gene is located in intron of *Gm35188* gene, the partial sequence of intron of *Gm35188* gene will be deleted together in this strategy.
- The effect on *Arhgap5-201* is unknown.
- The *Arhgap5* gene is located on the Chr12. 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)

Arhgap5 Rho GTPase activating protein 5 [*Mus musculus* (house mouse)]

Gene ID: 11855, updated on 12-Aug-2019

Summary

Official Symbol Arhgap5 provided by [MGI](#)
Official Full Name Rho GTPase activating protein 5 provided by [MGI](#)
Primary source [MGI:MGI:1332637](#)
See related [Ensembl:ENSMUSG00000035133](#)
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 p190B; p190-B
Expression Broad expression in cerebellum adult (RPKM 9.8), frontal lobe adult (RPKM 6.6) and 21 other tissues [See more](#)
Orthologs [human](#) [all](#)

Genomic context

Location: 12 C1; 12 22.16 cM

See Arhgap5 in [Genome Data Viewer](#)

Exon count: 8

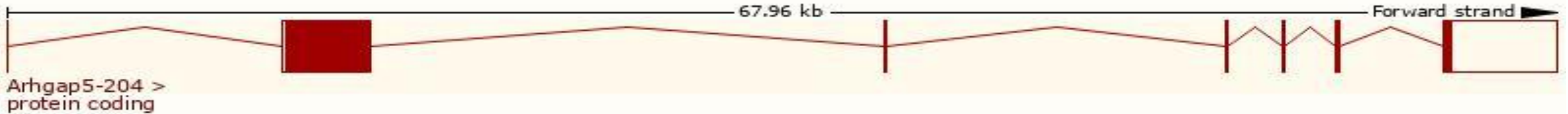
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	12	NC_000078.6 (52503763..52571978)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	12	NC_000078.5 (53617064..53668839)

Transcript information (Ensembl)

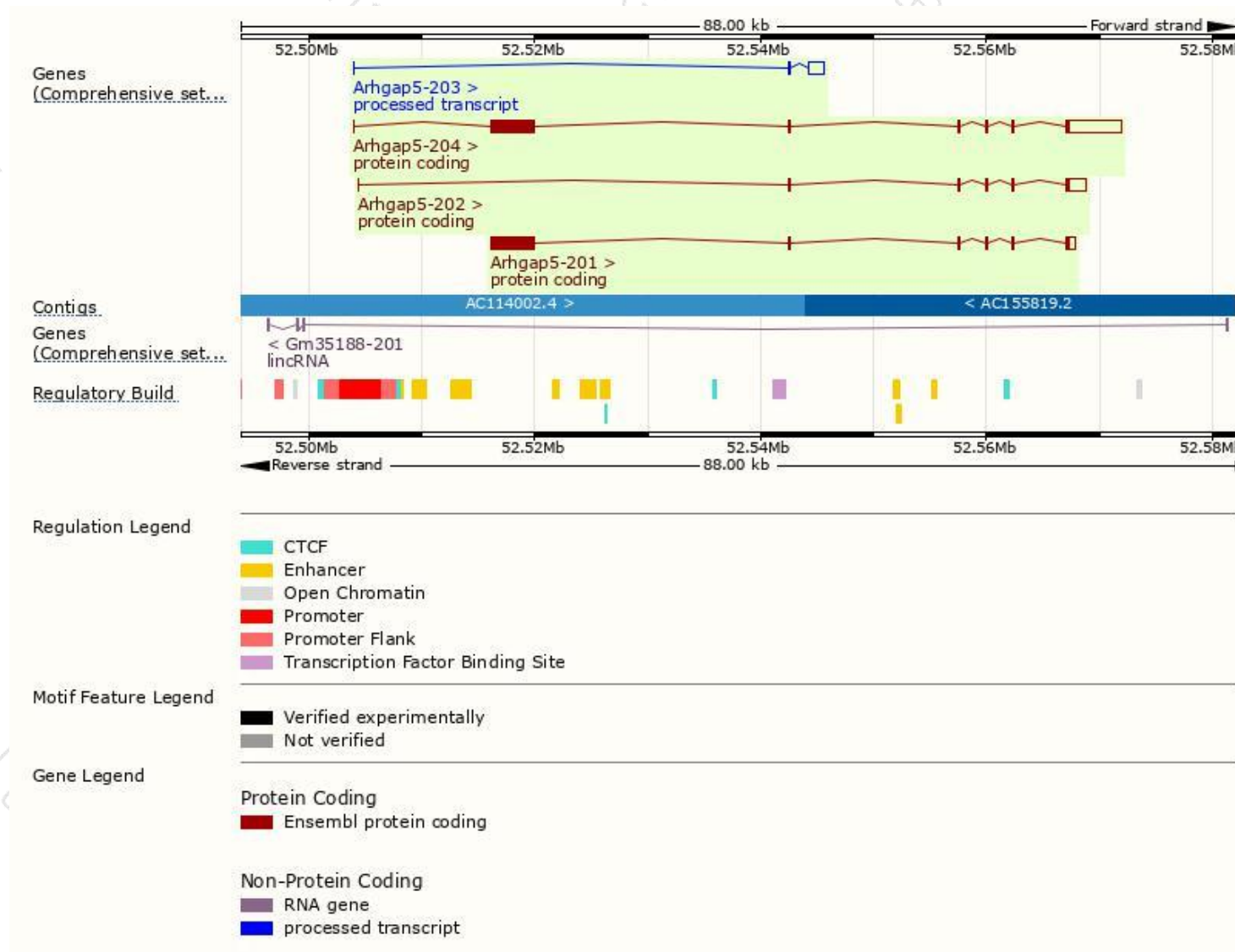
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arhgap5-204	ENSMUST00000219443.1	9323	1503aa	Protein coding	CCDS36443	E9PYT0	TSL:5 GENCODE basic APPRIS P1
Arhgap5-201	ENSMUST00000110725.1	5197	1503aa	Protein coding	CCDS36443	E9PYT0	TSL:1 GENCODE basic APPRIS P1
Arhgap5-202	ENSMUST00000217820.1	2332	241aa	Protein coding	-	A0A1W2P6H9	TSL:5 GENCODE basic
Arhgap5-203	ENSMUST00000218755.1	1669	No protein	Processed transcript	-	-	TSL:1

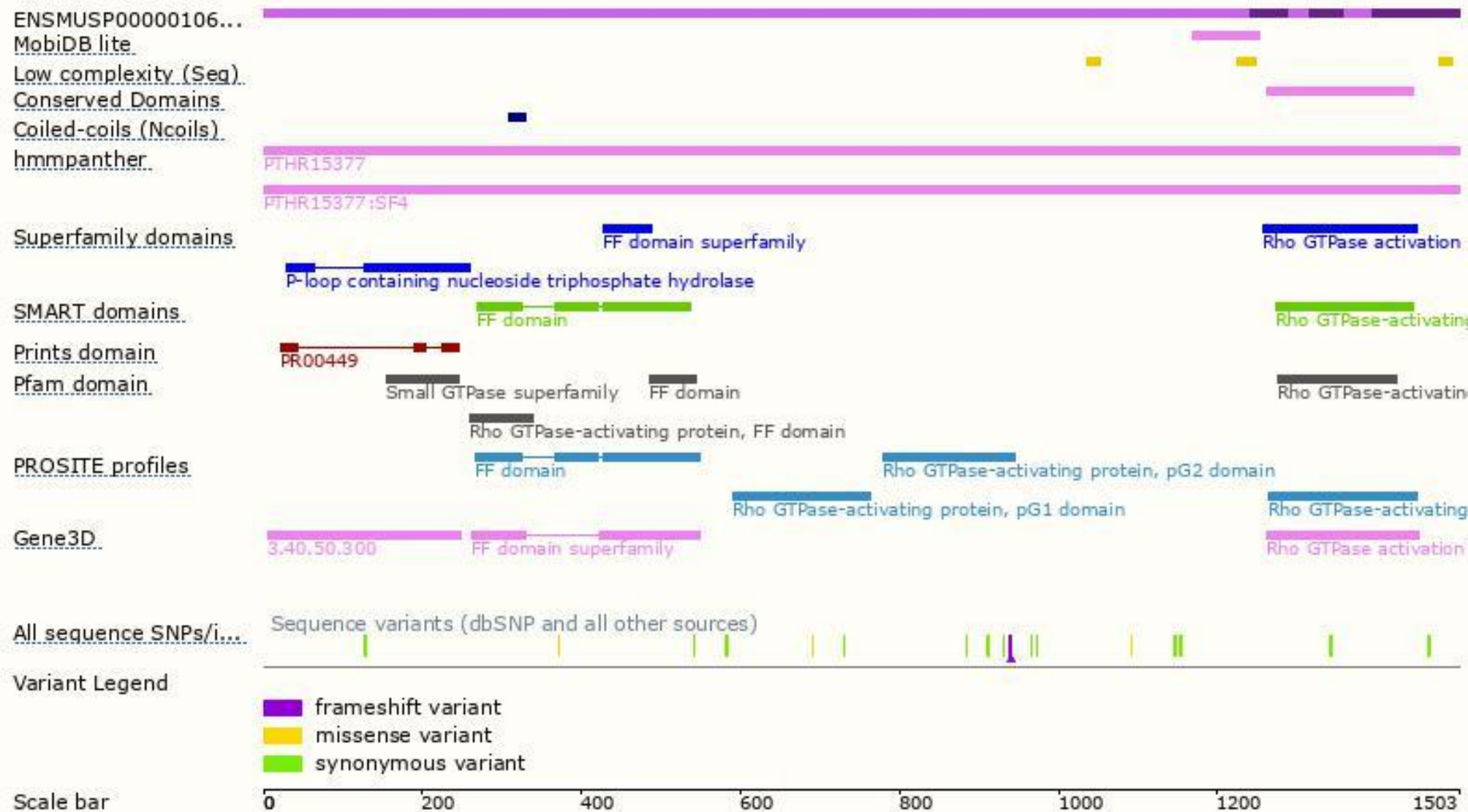
The strategy is based on the design of *Arhgap5-204* transcript,The transcription is shown below



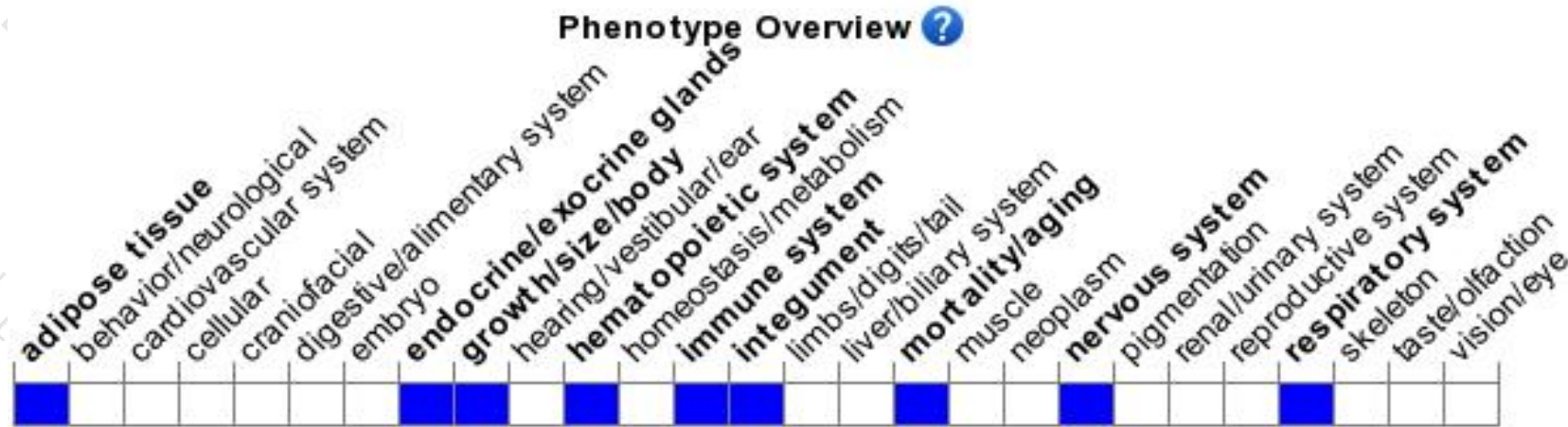
Genomic location distribution



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, Homozygotes die at birth, are 30% smaller, do not inflate their lungs, and show a small thymus, abnormal adipocyte differentiation and brain defects in the corpus callosum, anterior commissure and lateral ventricles. Mutant MEFs show impaired adipogenesis but undergo myogenesis in response to IGF-1.

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

Tel: 400-9660890

