Abcg5 Cas9-CKO Strategy

Designer: Huan Wang

Reviewer: Huan Fan

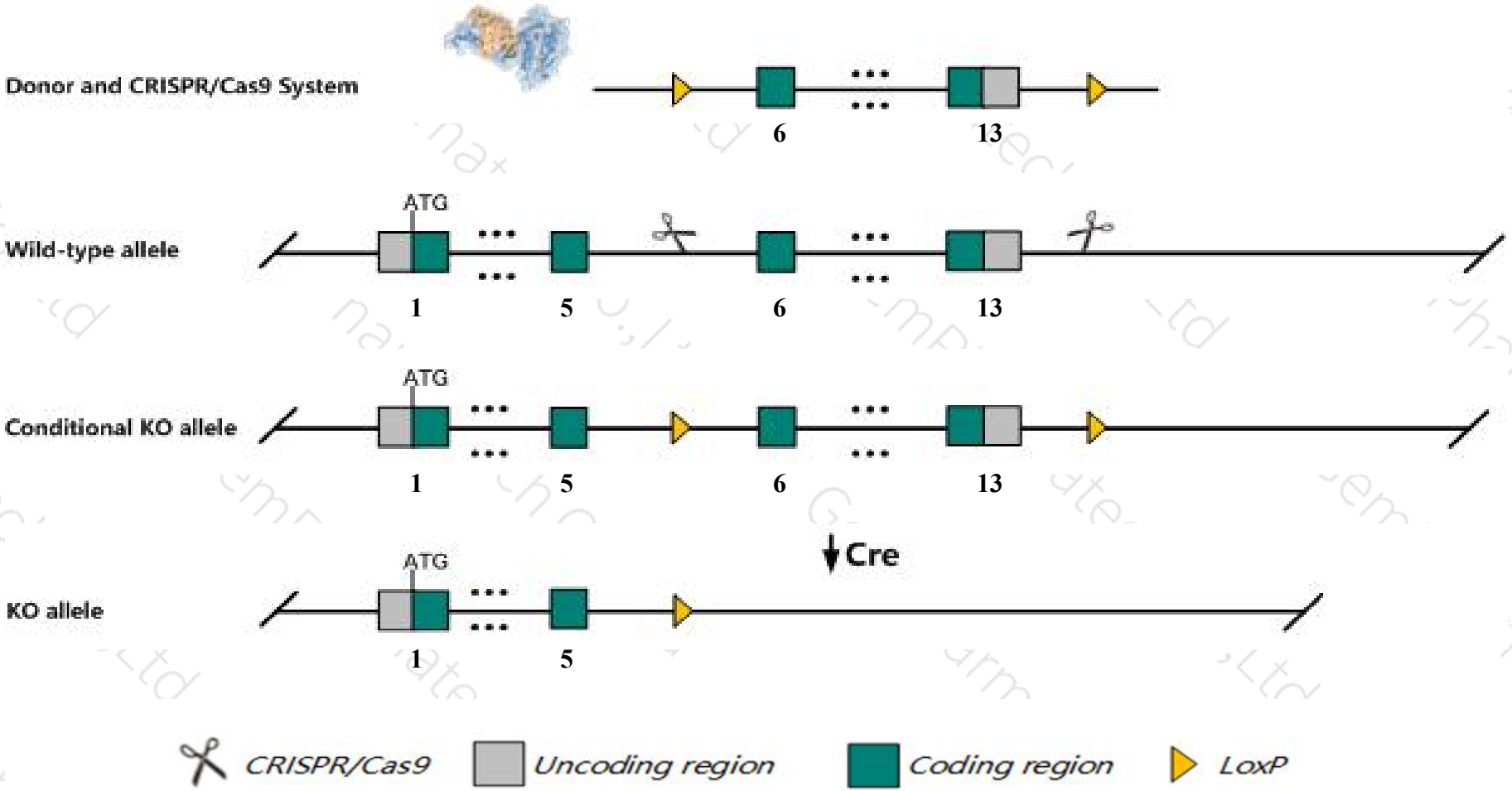
Design Date: 2020-3-9

Project Overview

Project Name	***Abcg5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Abcg5* gene. The schematic diagram is as follows:



Technical routes

- The *Abcg5* gene has 4 transcripts. According to the structure of *Abcg5* gene, exon6-exon13 of *Abcg5-201* (ENSMUST00000066175.9) transcript is recommended as the knockout region.The region contains most coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Abcg5* 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,Homozygotes for a null allele show hyperabsorption of dietary plant sterols and sitosterolemia. Spontaneous mutants are small, infertile and hunched and display anemia, leukopenia, macrothrombocytopenia, other hematologic defects, cardiomyopathy, high plasma phytosterol levels and premature death.

- The *Abcg5* gene is located on the Chr17. 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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Abcg5 ATP binding cassette subfamily G member 5 [Mus musculus (house mouse)]

Gene ID: 27409, updated on 7-Apr-2019

Summary

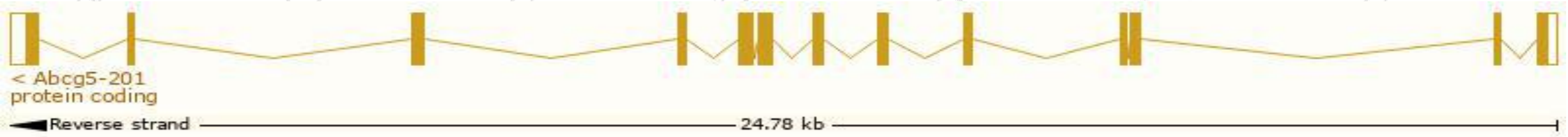
Official Symbol	Abcg5 provided by MGI
Official Full Name	ATP binding cassette subfamily G member 5 provided by MGI
Primary source	MGI:MGI:1351659
See related	Ensembl:ENSMUSG00000040505
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	cmp, sterolin-1, trac
Summary	The protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the White subfamily, and functions as a half-transporter to limit intestinal absorption and promote biliary excretion of sterols. Disruption of this gene in mice results in thrombocytopenia, prolonged bleeding times, anemia, leukopenia, infertility, shortened life span and cardiomyopathy. Mice lacking this gene show symptoms of sitosterolemia. [provided by RefSeq, Nov 2015]
Expression	Biased expression in duodenum adult (RPKM 83.5), large intestine adult (RPKM 69.9) and 2 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

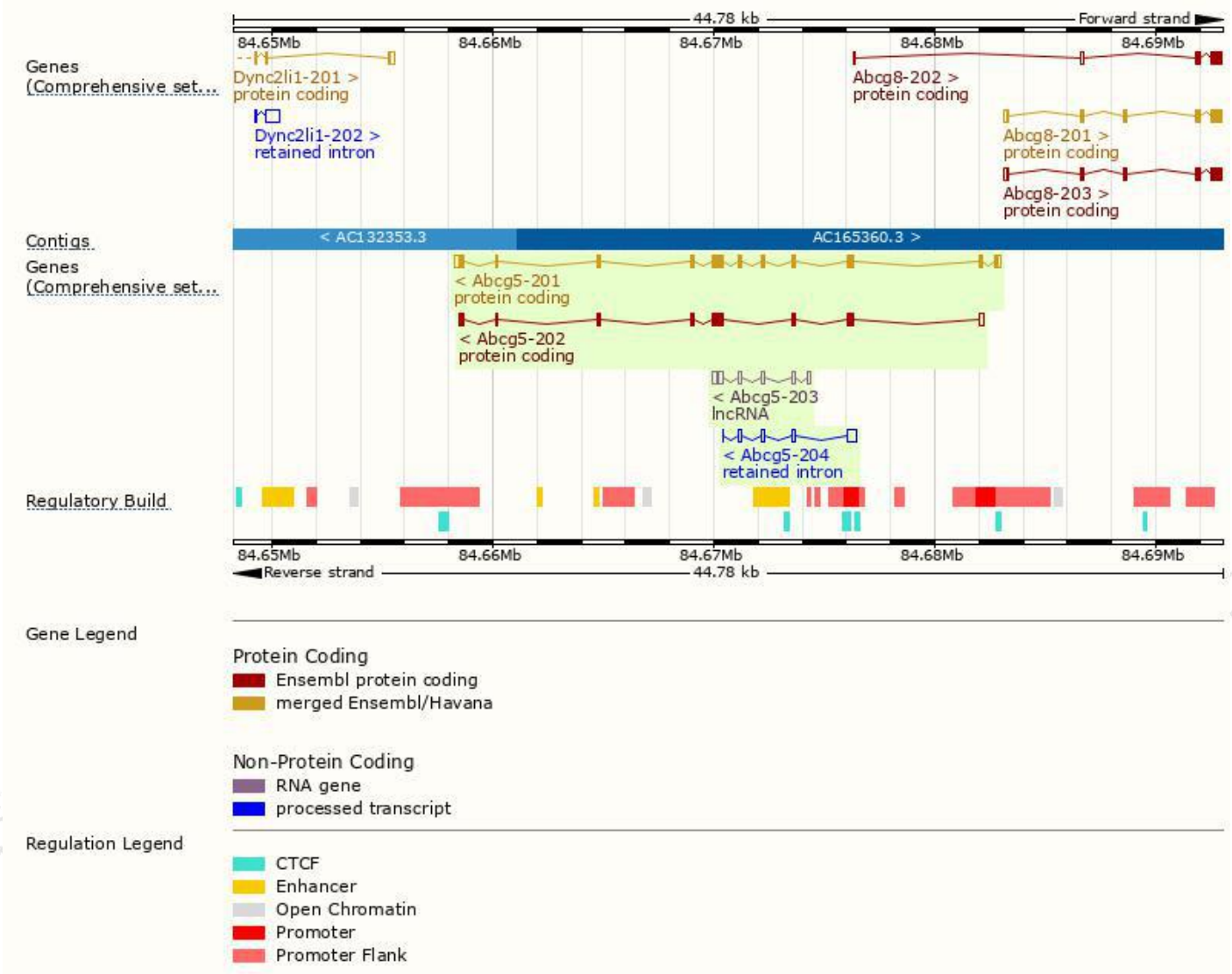
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Abcg5-201	ENSMUST00000066175.9	2351	652aa	Protein coding	CCDS29001	Q99PE8	TSL:1 GENCODE basic APPRIS P1
Abcg5-202	ENSMUST00000163375.1	1613	480aa	Protein coding	-	E9QA91	TSL:5 GENCODE basic
Abcg5-204	ENSMUST00000172439.1	850	No protein	Retained intron	-	-	TSL:5
Abcg5-203	ENSMUST00000171544.7	944	No protein	lncRNA	-	-	TSL:5

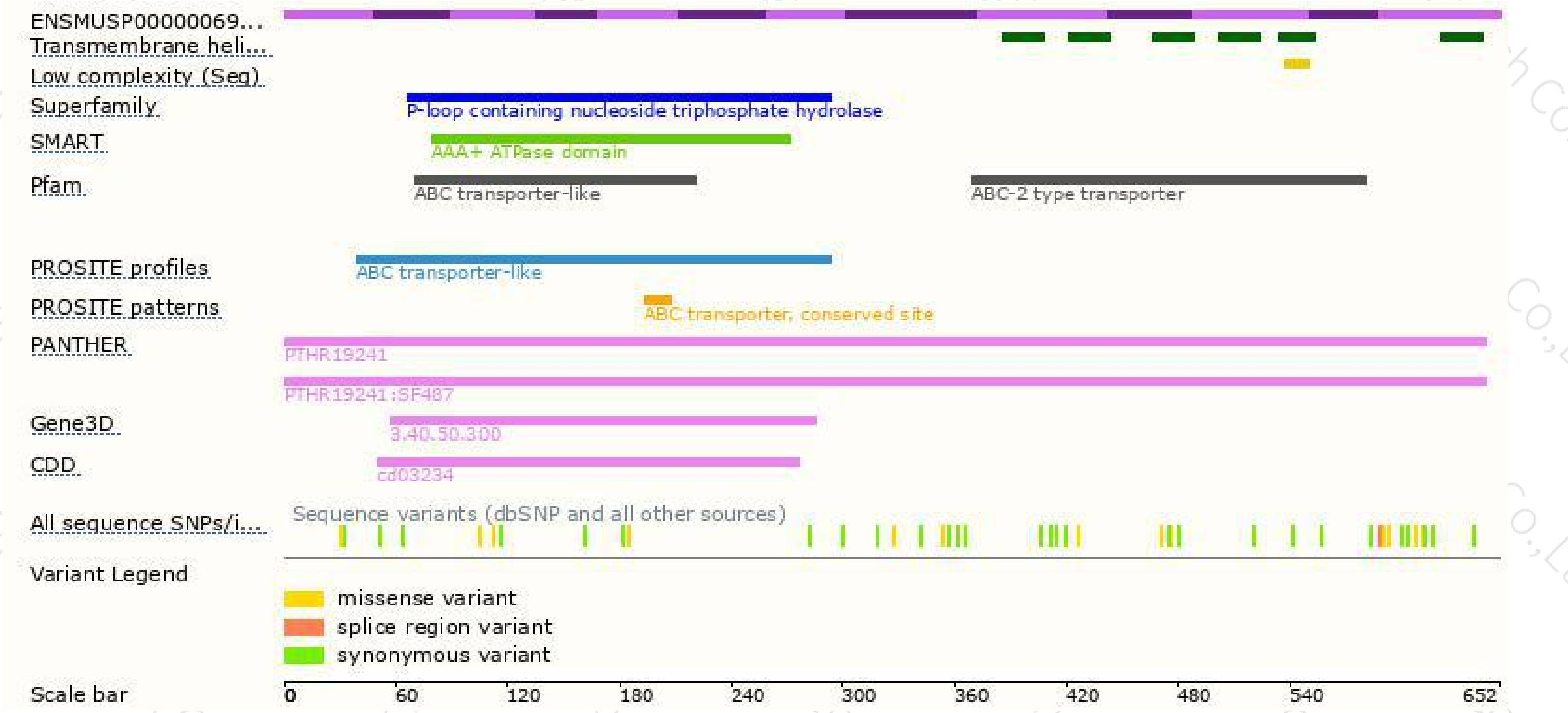
The strategy is based on the design of *Abcg5-201* transcript,The transcription is shown below



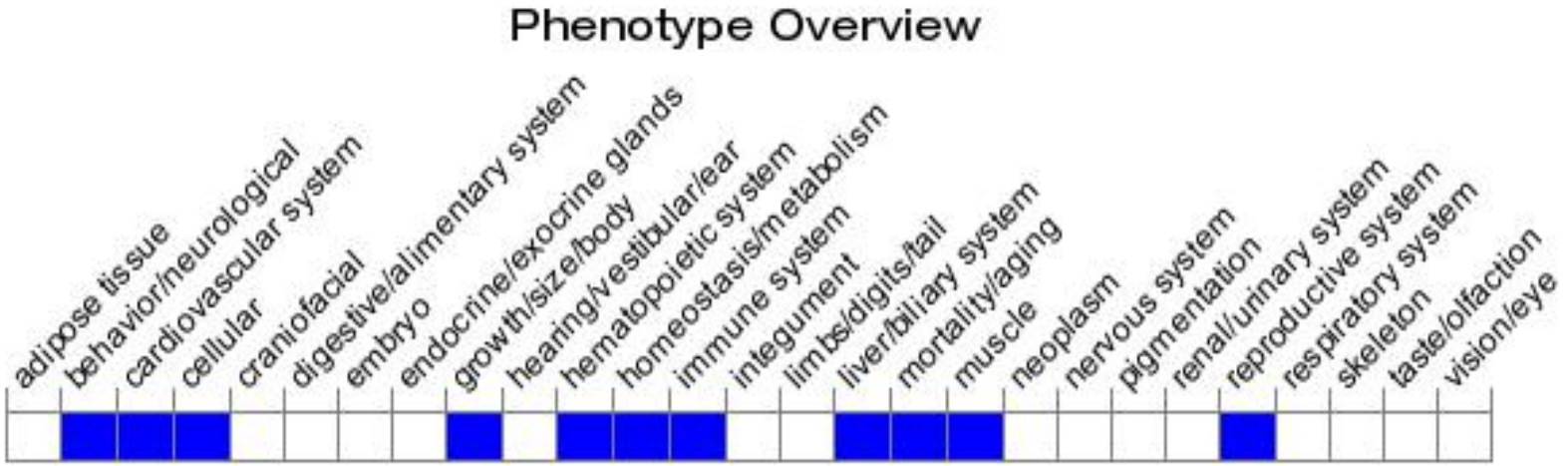
Genomic location distribution



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.

Tel: 400-9660890

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