Crk Cas9-CKO Strategy

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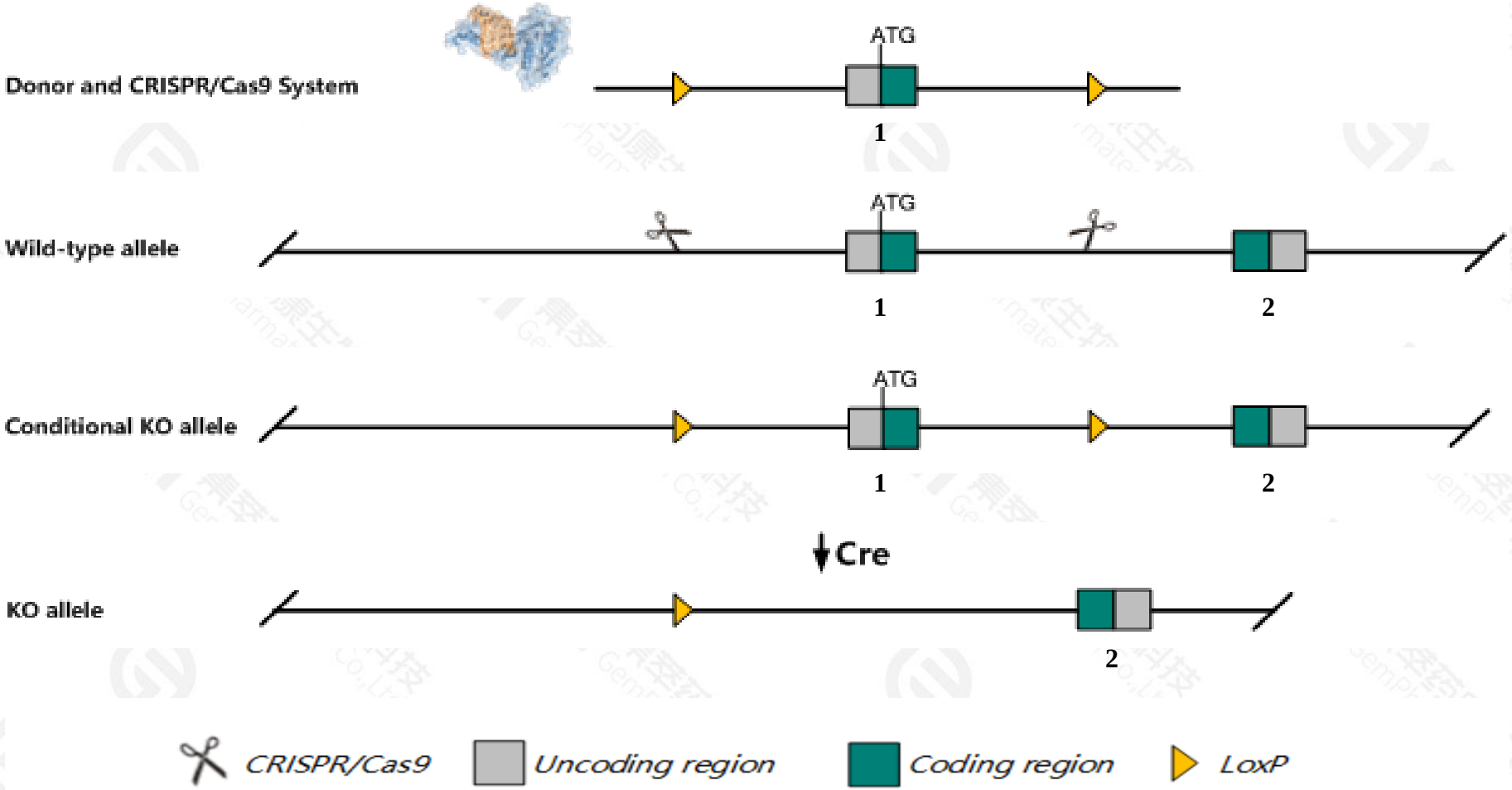
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Crk* gene has 5 transcripts. According to the structure of *Crk* gene, exon1 of *Crk-204*(ENSMUST00000108426.8) 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 *Crk* 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 was 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 an isoform specific knockout do not exhibit any obvious abnormalities. Mice homozygous of a null allele of both isoforms exhibit fetal and perinatal lethality associated with abnormal cardiovascular morphology.

➢ The *Crk* gene is located on the Chr11. 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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Crk v-crk avian sarcoma virus CT10 oncogene homolog [Mus musculus (house mouse)]

Gene ID: 12928, updated on 13-Mar-2020

Summary

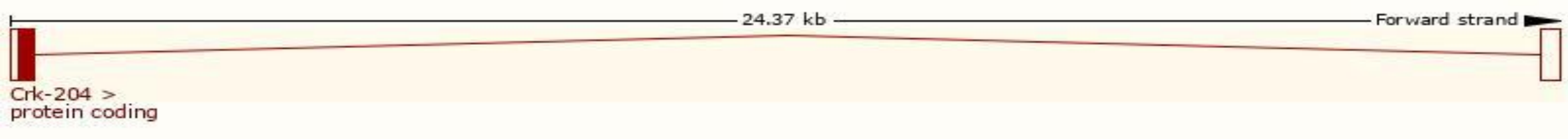
Official Symbol	Crk provided by MGI
Official Full Name	v-crk avian sarcoma virus CT10 oncogene homolog provided byMGI
Primary source	MGI:MGI:88508
See related	Ensembl:ENSMUSG00000017776
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Crk-I, Crk-II, Crk-III, Crk3, CrkIII, Crko, c-Crk, p38
Summary	This gene is part of a family of adapter proteins that mediate formation of signal transduction complexes in response to extracellular stimuli, such as growth and differentiation factors. Protein-protein interactions occur through the SH2 domain, which binds phosphorylated tyrosine residues, and the SH3 domain, which binds proline-rich peptide motifs. These interactions promote recruitment and activation of effector proteins to regulate cell migration, adhesion, and proliferation. In mouse this protein is essential for embryonic development. Alternatively spliced transcripts encoding different isoforms with distinct biological activity have been described. [provided by RefSeq, Mar 2013]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 18.5), bladder adult (RPKM 18.1) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

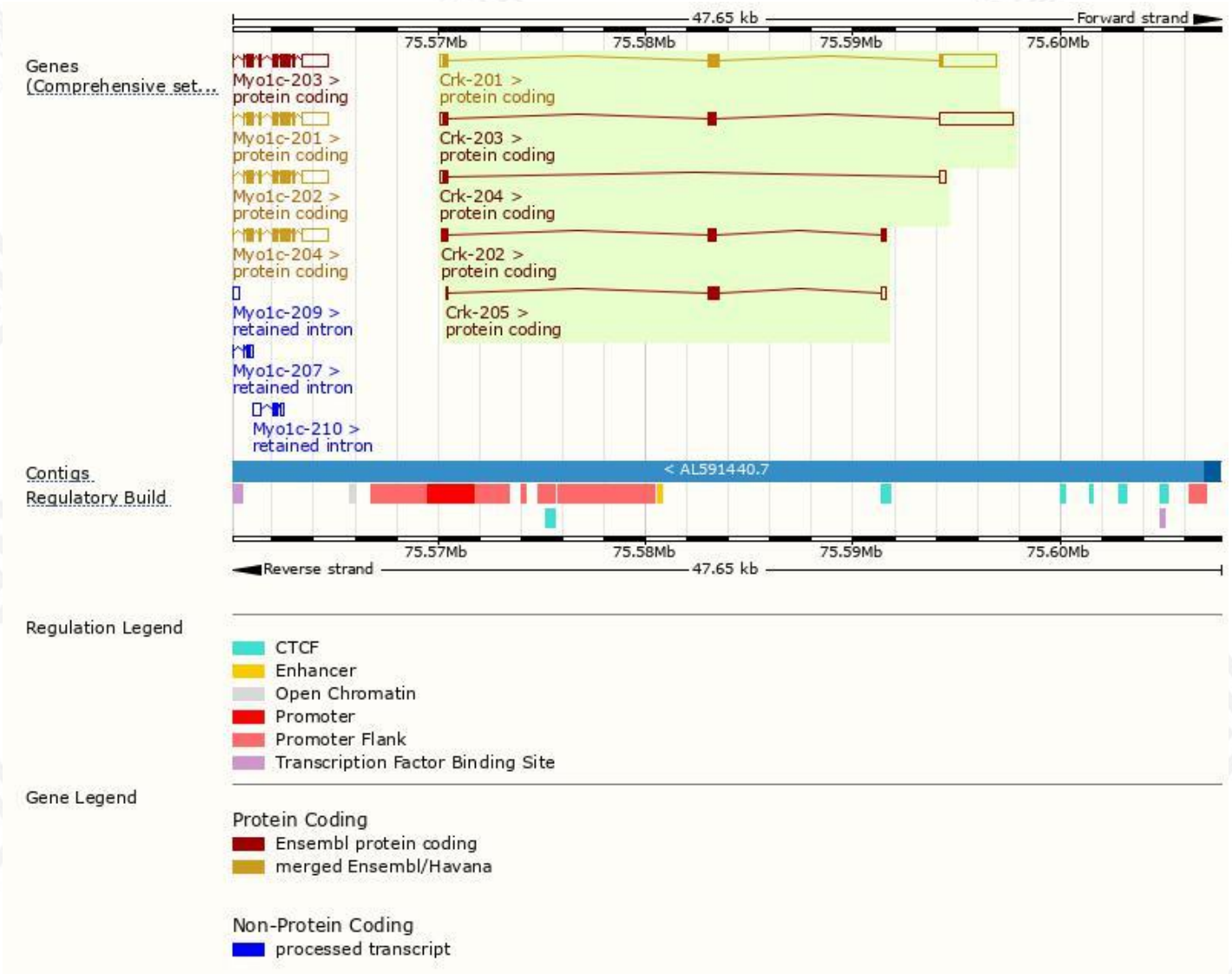
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Crk-203	ENSMUST00000108425.7	4290	204aa	Protein coding	CCDS70241	Q8JZR2	TSL:1 GENCODE basic
Crk-201	ENSMUST00000017920.13	3642	304aa	Protein coding	CCDS25055	Q5ND51 Q64010	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Crk-204	ENSMUST00000108426.7	671	82aa	Protein coding	CCDS70242	Q3TQV3	TSL:3 GENCODE basic
Crk-202	ENSMUST00000093115.3	965	261aa	Protein coding	-	Q5ND50	TSL:1 GENCODE basic
Crk-205	ENSMUST00000147718.1	852	224aa	Protein coding	-	F7D232	CDS 5' incomplete TSL:3

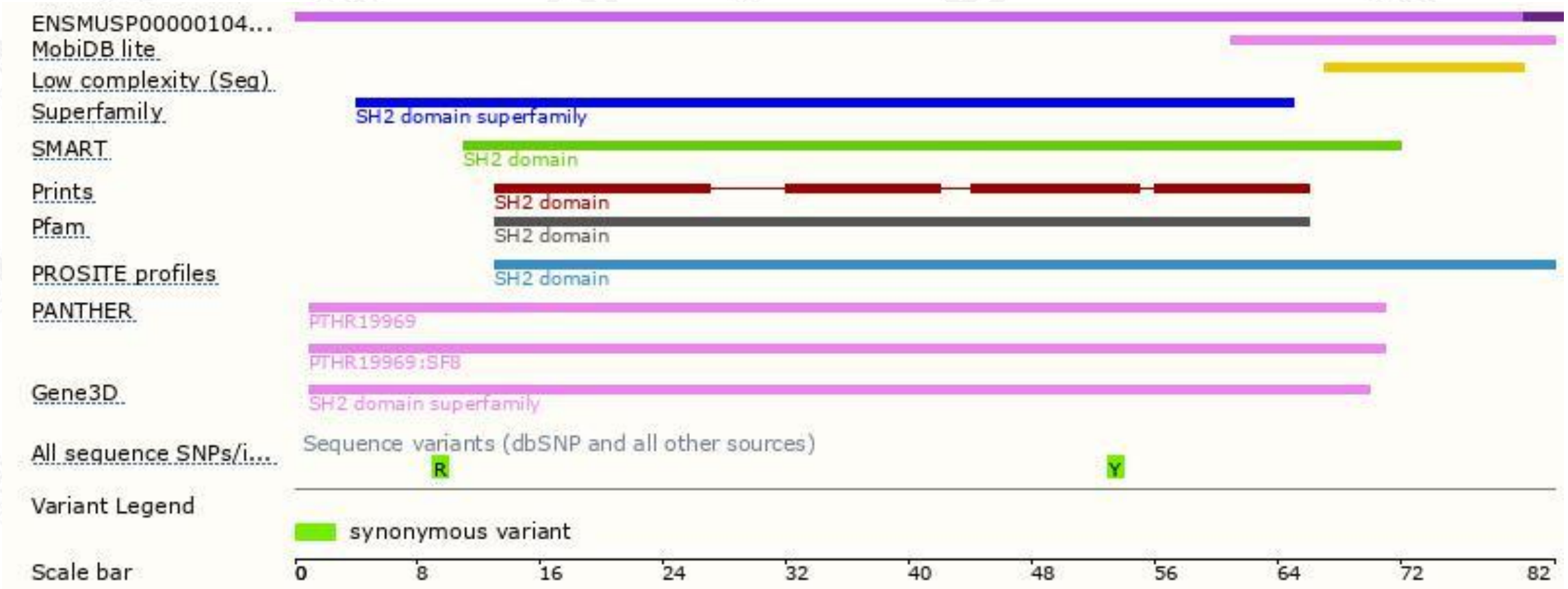
The strategy is based on the design of *Crk-204* transcript,the transcription is shown below:



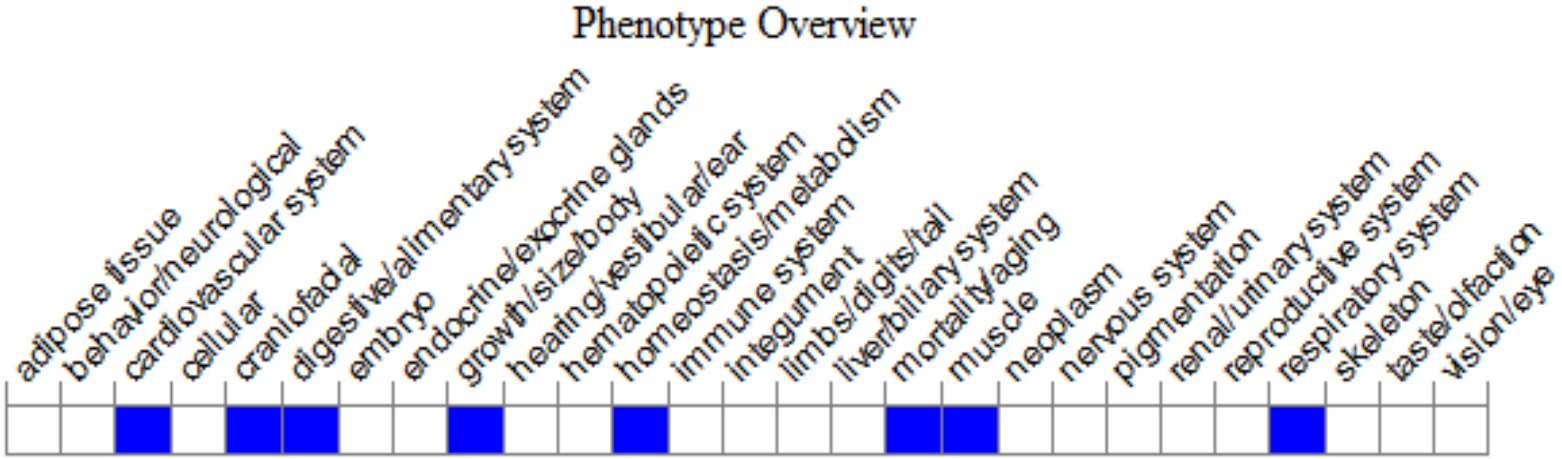
Genomic location distribution



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/).

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

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