Ngf Cas9-CKO Strategy

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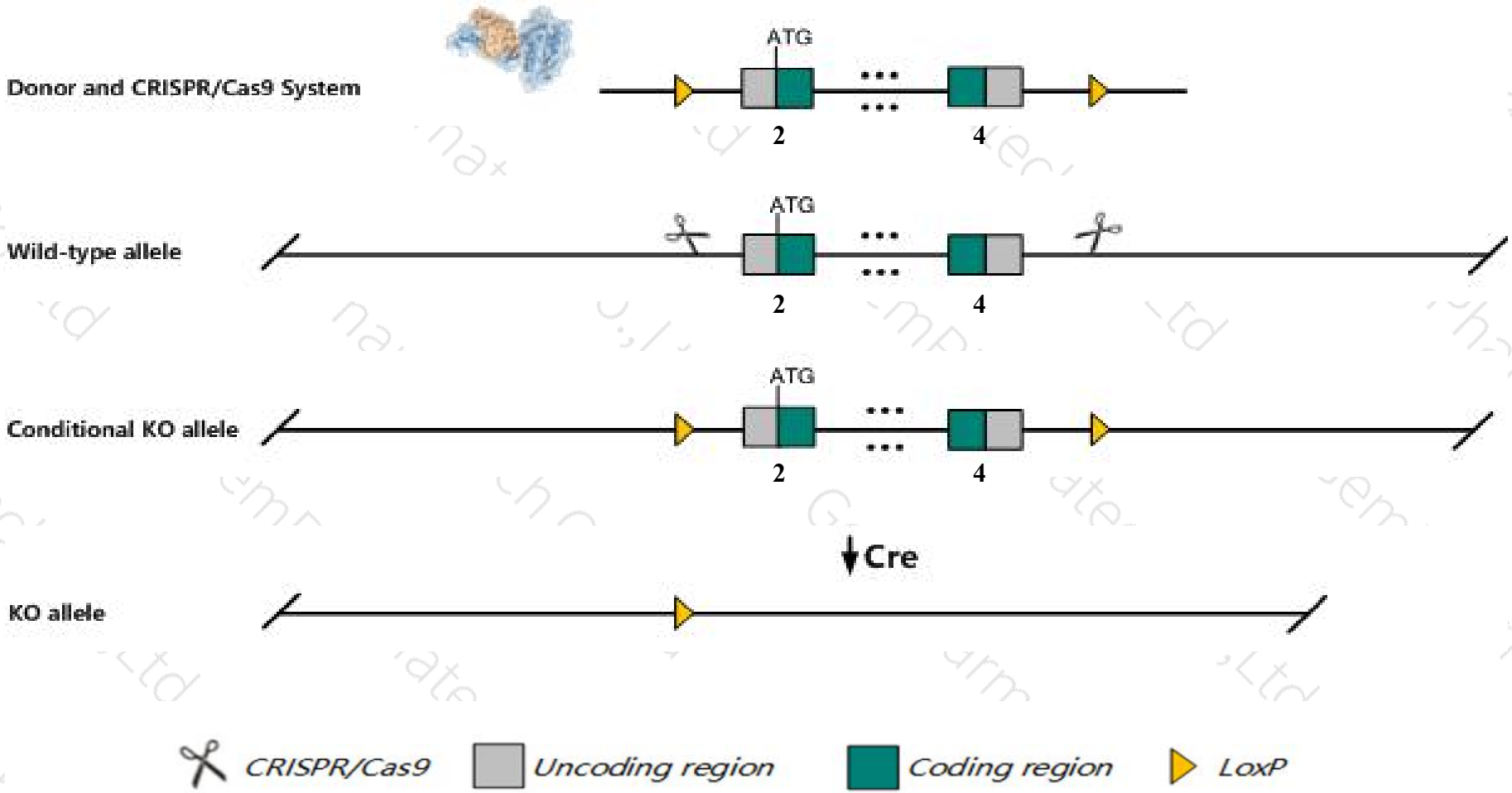
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Ngf* gene has 4 transcripts. According to the structure of *Ngf* gene, exon2-exon4 of *Ngf-202*(ENSMUST00000106925.8) 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 *Ngf* 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.

Notice

➢ According to the existing MGI data,homozygous null mutants exhibit elevated pain threshold, loss of neurons in both sensory and sympathetic ganglia, but diminished apoptosis in the retina and spinal cord. Heterozygotes exhibit a substantially reduced number of sympathetic neurons.

➢ The *Ngf* gene is located on the Chr3. 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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Ngf nerve growth factor [Mus musculus (house mouse)]

Gene ID: 18049, updated on 13-Mar-2020

Summary

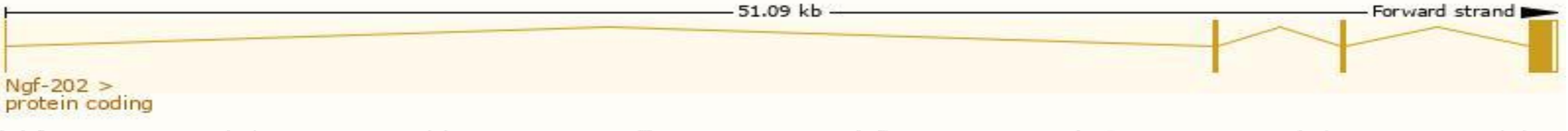
Official Symbol	Ngf provided by MGI
Official Full Name	nerve growth factor provided byMGI
Primary source	MGI:MGI:97321
See related	Ensembl:ENSMUSG00000027859
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Ngfb, beta-NGF
Expression	Broad expression in limb E14.5 (RPKM 9.7), subcutaneous fat pad adult (RPKM 5.1) and 20 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

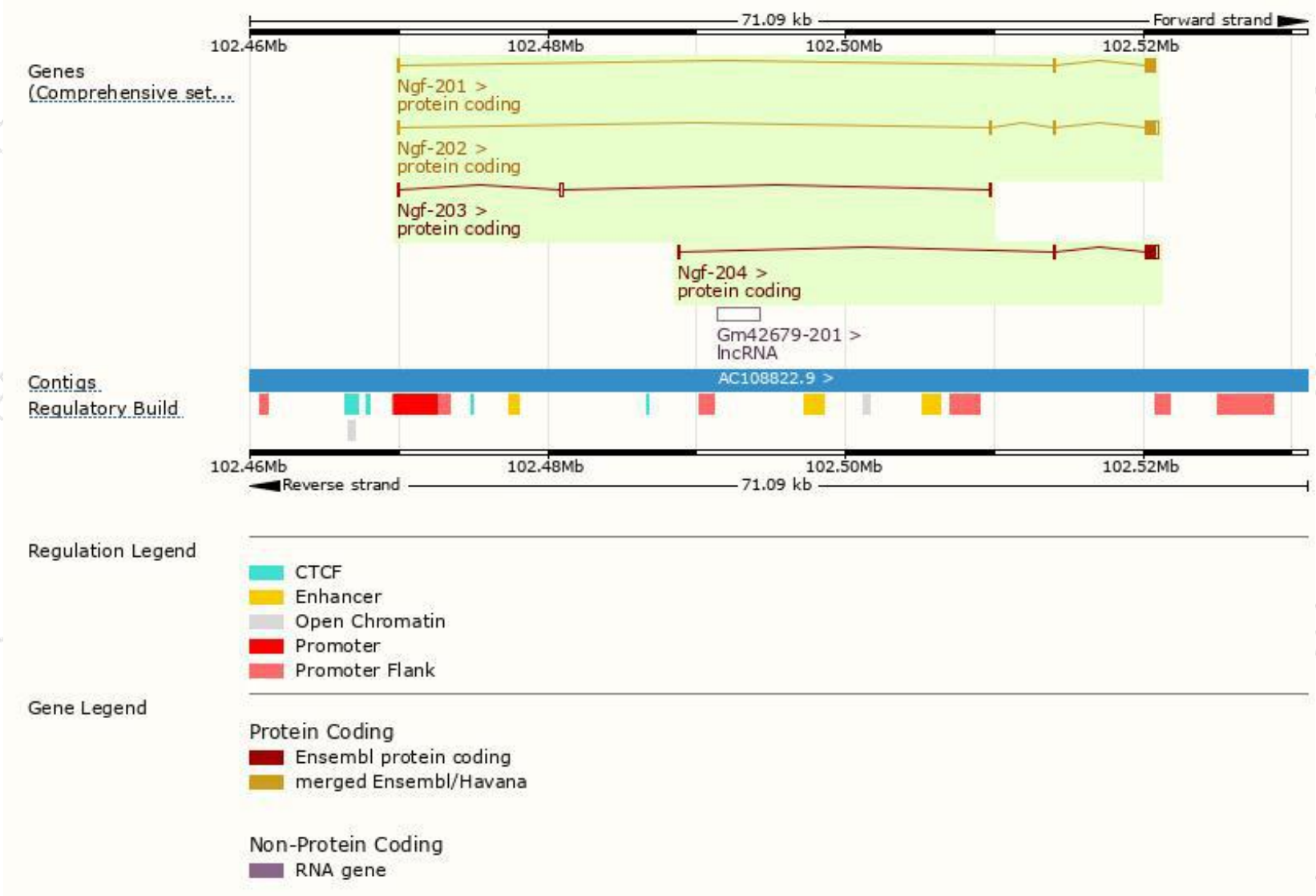
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ngf-202	ENSMUST00000106925.8	1187	307aa	Protein coding	CCDS17687	Q6LDU8	TSL:1 GENCODE basic
Ngf-201	ENSMUST00000035952.4	904	241aa	Protein coding	CCDS51025	P01139	TSL:2 GENCODE basic APPRIS P1
Ngf-204	ENSMUST00000198644.1	1120	303aa	Protein coding	-	A0A0G2JEK0	TSL:5 GENCODE basic
Ngf-203	ENSMUST00000198168.4	322	22aa	Protein coding	-	A0A0G2JG51	CDS 3' incomplete TSL:2

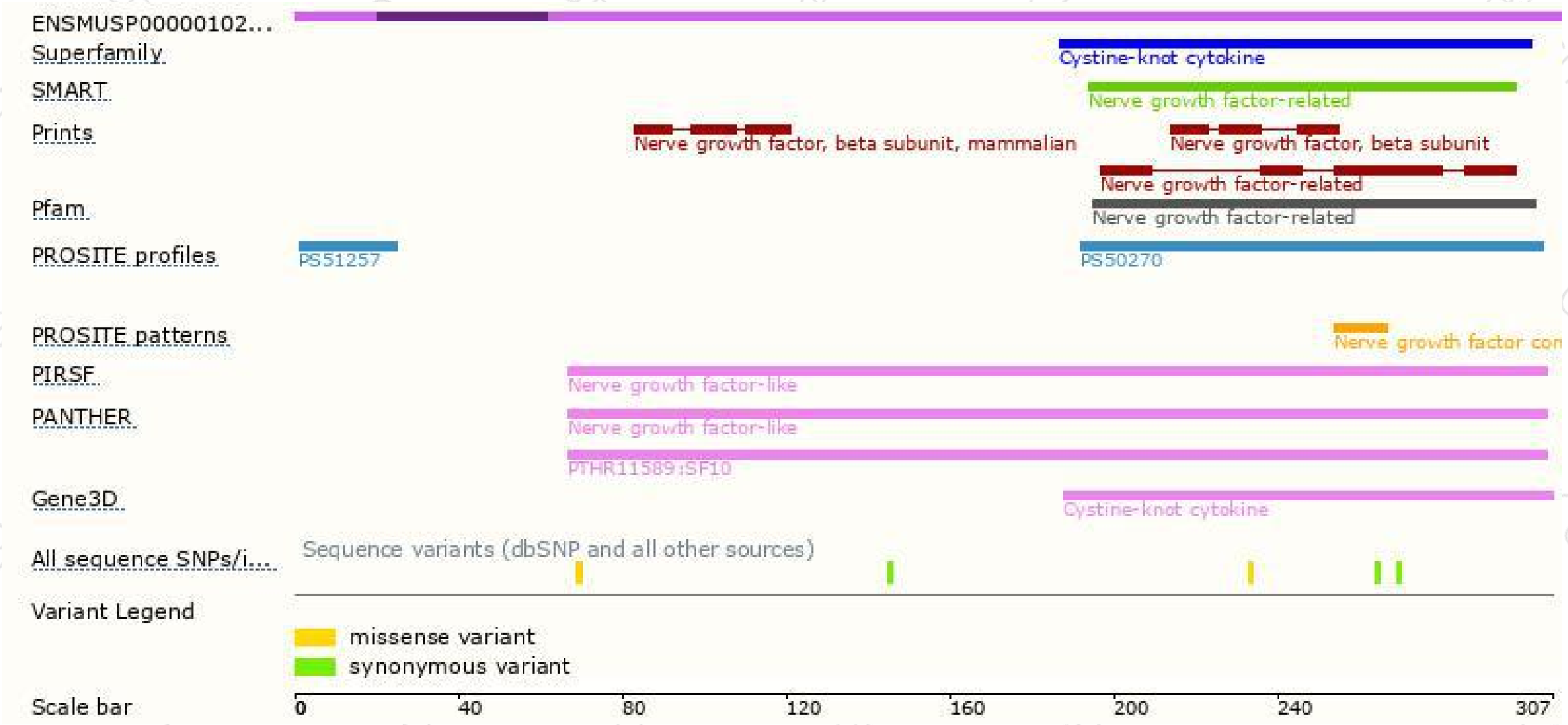
The strategy is based on the design of *Ngf-202* transcript,the transcription is shown below:



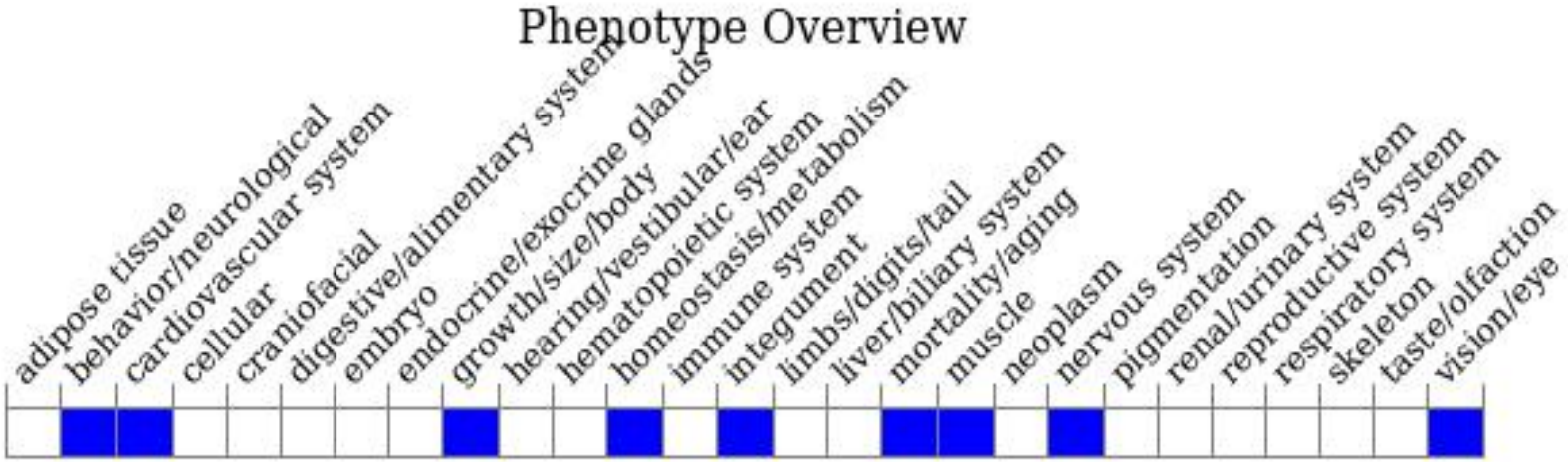
Genomic location distribution



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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