Arhgap33 Cas9-CKO Strategy

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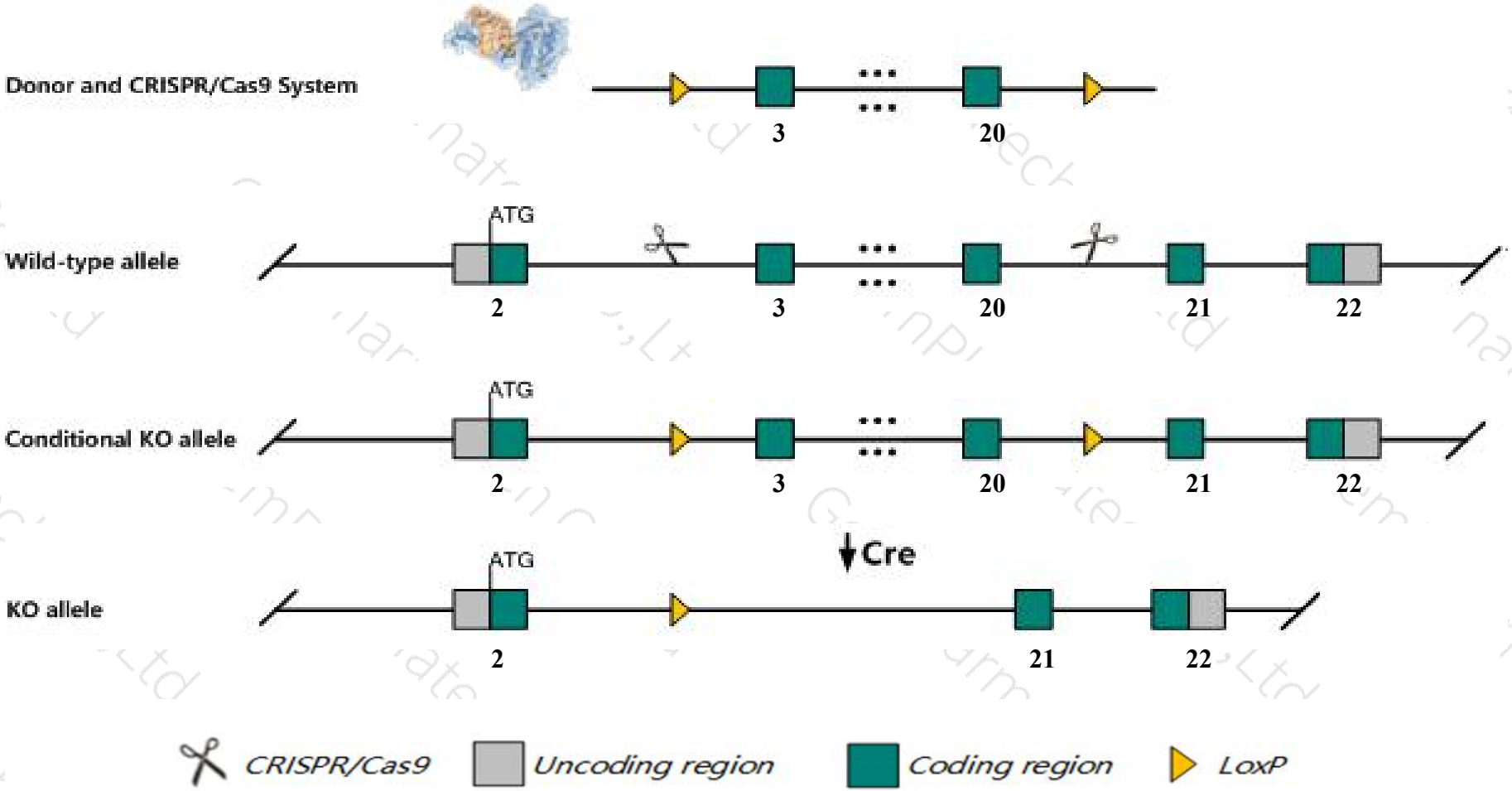
Project Overview

Project Name	***Arhgap33***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Arhgap33* gene. The schematic diagram is as follows:



Technical routes

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- The *Arhgap33* gene has 8 transcripts. According to the structure of *Arhgap33* gene, exon3-exon20 of *Arhgap33-204* (ENSMUST00000207860.1) transcript is recommended as the knockout region.The region contains 1936bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Arhgap33* 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,Mice homozygous for a null mutation display region specific thinning of the cerebral cortex with reduced dendritic complexity.

➢ The *Arhgap33* 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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Arhgap33 Rho GTPase activating protein 33 [*Mus musculus* (house mouse)]

Gene ID: 233071, updated on 12-Aug-2019

Summary

Official Symbol	Arhgap33 provided by MGI
Official Full Name	Rho GTPase activating protein 33 provided by MGI
Primary source	MGI:MGI:2673998
See related	Ensembl:ENSMUSG00000036882
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	Snx26; TCGAP; AW324378; NOMA-GAP
Expression	Broad expression in whole brain E14.5 (RPKM 45.7), CNS E14 (RPKM 41.4) and 16 other tissues See more
Orthologs	human all

Genomic context

Location: 7; 7 B1

See Arhgap33 in Genome Data Viewer

Exon count: 23

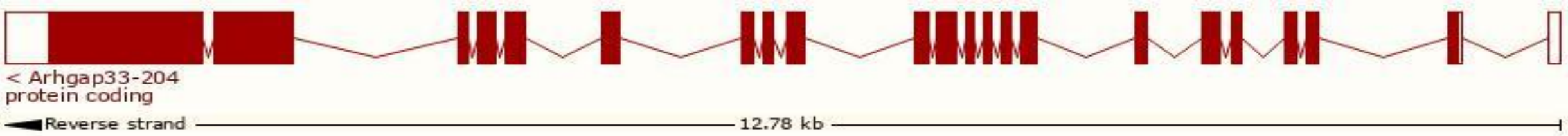
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	7	NC_000073.6 (30522225..30535171, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	7	NC_000073.5 (31307245..31320028, complement)

Transcript information（Ensembl）

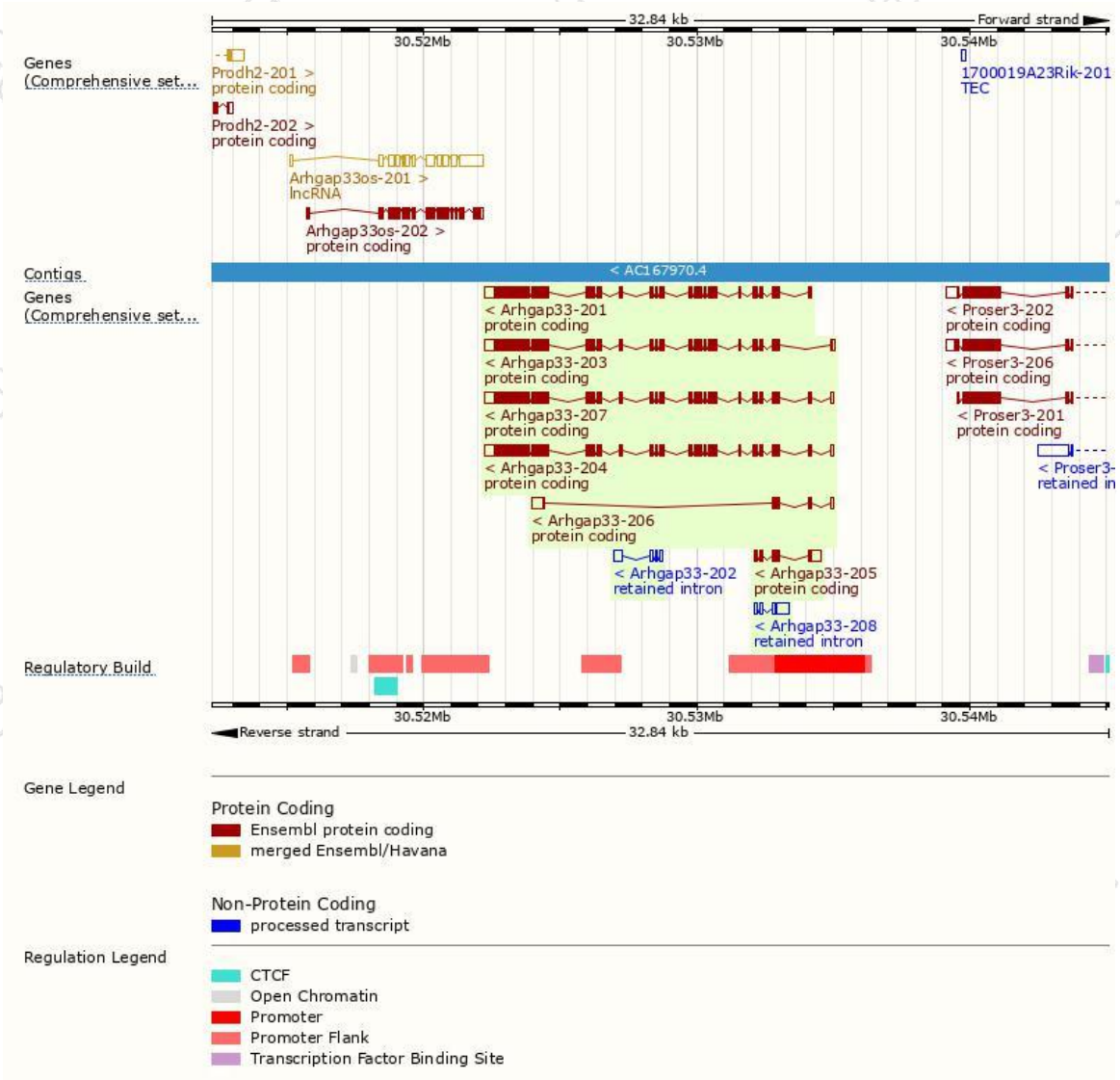
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arhgap33-204	ENSMUST00000207860.1	4396	1305aa	Protein coding	CCDS21095	Q80YF9	TSL:1 GENCODE basic APPRIS P3
Arhgap33-207	ENSMUST00000208538.1	4372	1305aa	Protein coding	CCDS21095	Q80YF9	TSL:1 GENCODE basic APPRIS P3
Arhgap33-203	ENSMUST00000207858.1	4337	1281aa	Protein coding	CCDS85264	A0A140LHW2	TSL:1 GENCODE basic APPRIS ALT2
Arhgap33-201	ENSMUST00000044338.4	4280	1305aa	Protein coding	CCDS21095	Q80YF9	TSL:1 GENCODE basic APPRIS P3
Arhgap33-205	ENSMUST00000208491.1	822	145aa	Protein coding	-	A0A140LIY1	CDS 3' incomplete TSL:3
Arhgap33-206	ENSMUST00000208522.1	781	97aa	Protein coding	-	A0A140LIC8	TSL:3 GENCODE basic
Arhgap33-208	ENSMUST00000208723.1	726	No protein	Retained intron	-	-	TSL:3
Arhgap33-202	ENSMUST00000207637.1	596	No protein	Retained intron	-	-	TSL:5

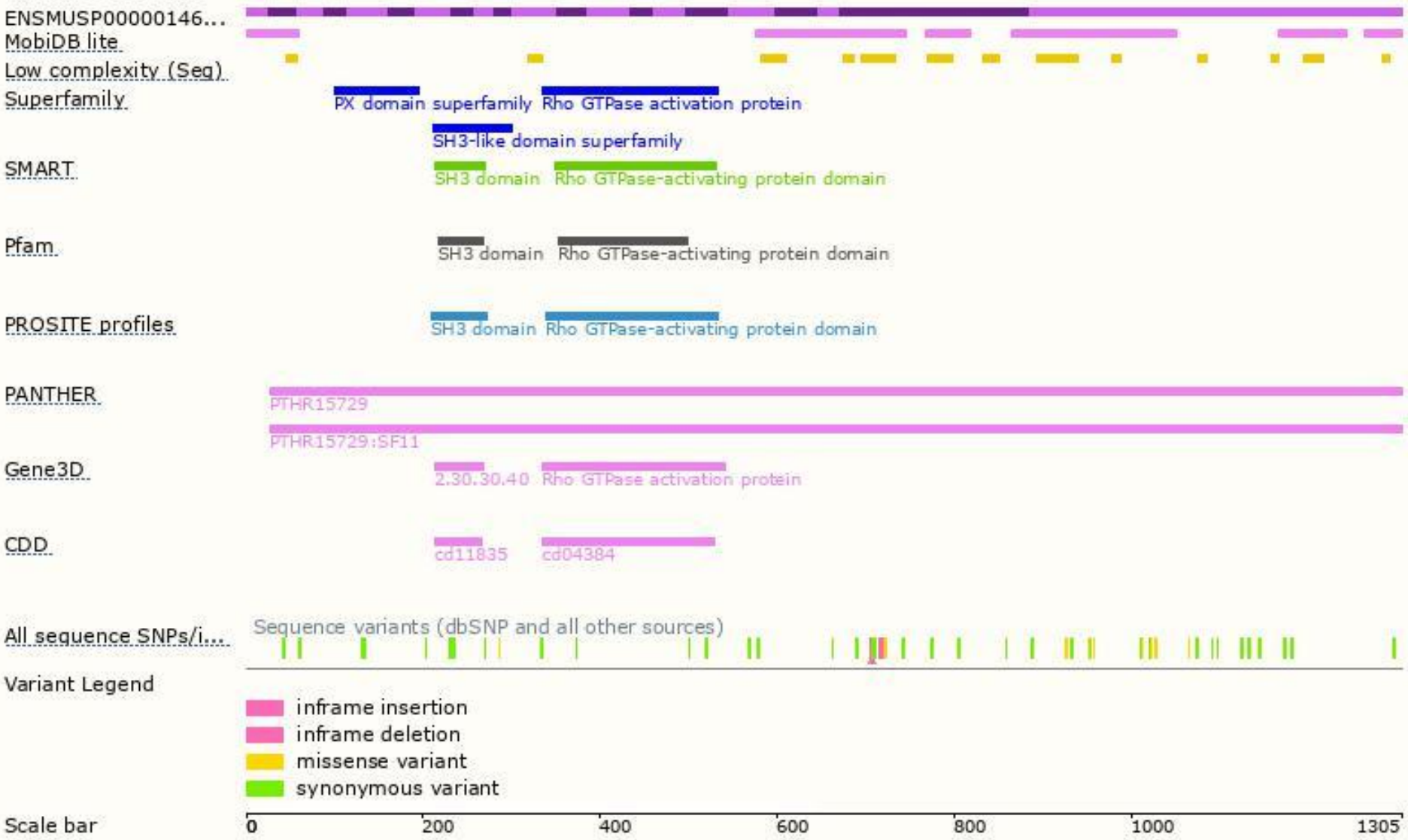
The strategy is based on the design of *Arhgap33-204* transcript,The transcription is shown below



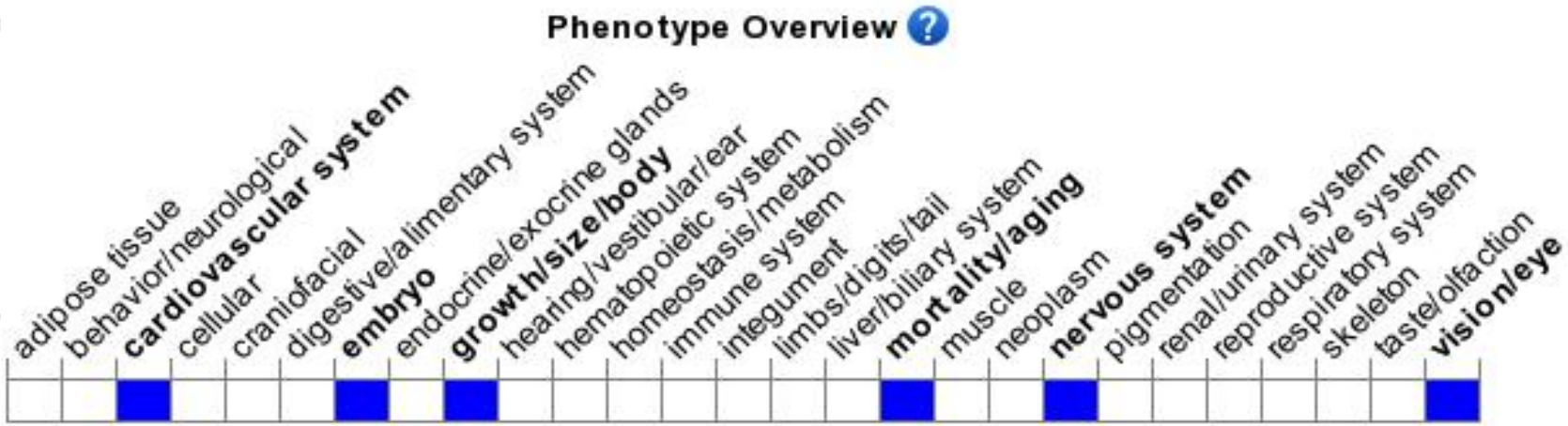
Genomic location distribution



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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