Spast Cas9-CKO Strategy

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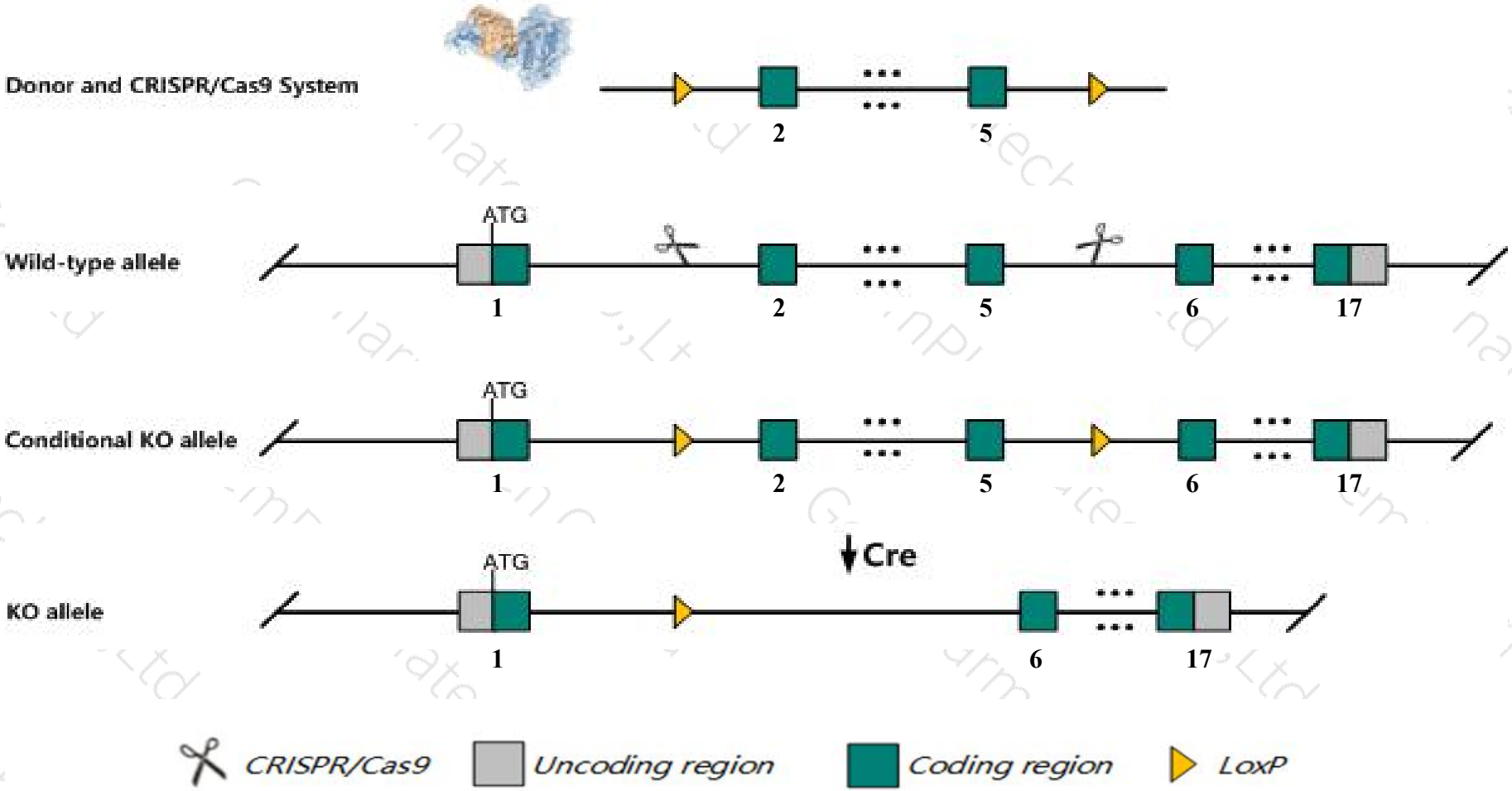
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Spast* gene has 5 transcripts. According to the structure of *Spast* gene, exon2-exon5 of *Spast-201* (ENSMUST00000024869.7) transcript is recommended as the knockout region.The region contains 455bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Spast* 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 mutation in this gene are sterile and display progressive axonopathy with focal axonal swellings and late onset gait abnormalities.

- The *Spast* 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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Spast spastin [Mus musculus (house mouse)]

Gene ID: 50850, updated on 25-Mar-2019

Summary

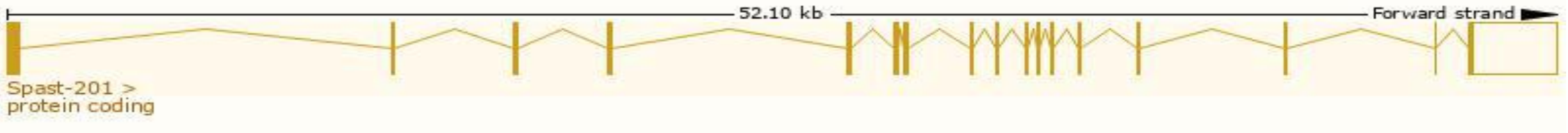
Official Symbol	Spast provided by MGI
Official Full Name	spastin provided by MGI
Primary source	MGI:MGI:1858896
See related	Ensembl:ENSMUSG00000024068
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Spg4, mKIAA1083
Expression	Broad expression in CNS E18 (RPKM 14.2), whole brain E14.5 (RPKM 14.2) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

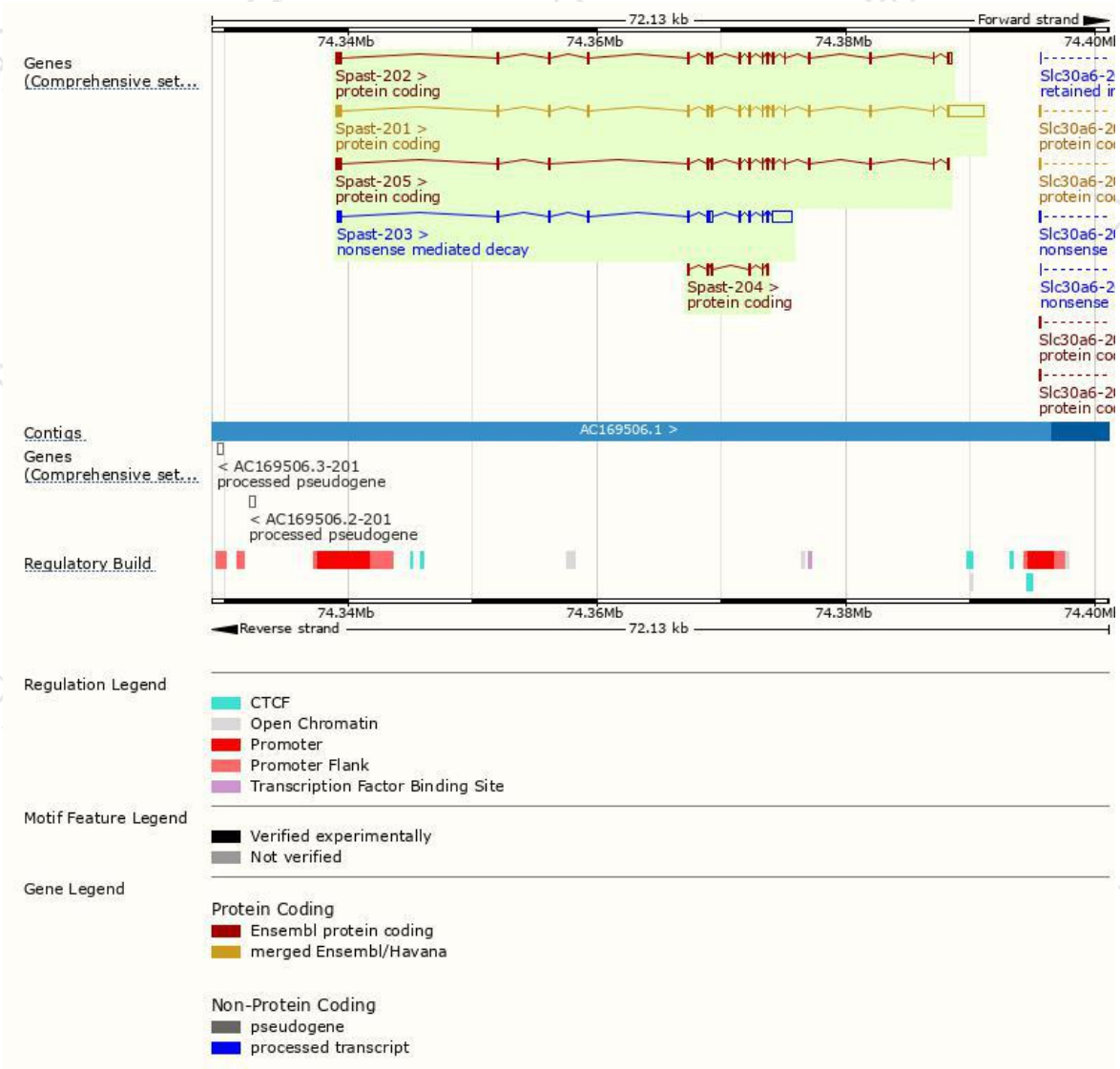
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spast-201	ENSMUST00000024869.7	4672	614aa	Protein coding	CCDS50181	Q9QYY8	TSL:1 GENCODE basic APPRIS P2
Spast-202	ENSMUST00000224711.1	2066	613aa	Protein coding	-	A0A286YE25	GENCODE basic APPRIS ALT2
Spast-205	ENSMUST00000233611.1	1746	581aa	Protein coding	-	A0A3B2WC92	GENCODE basic APPRIS ALT2
Spast-204	ENSMUST00000233554.1	573	191aa	Protein coding	-	A0A3B2WBA7	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete
Spast-203	ENSMUST00000225549.1	2897	300aa	Nonsense mediated decay	-	A0A286YCJ4	CDS 5' incomplete

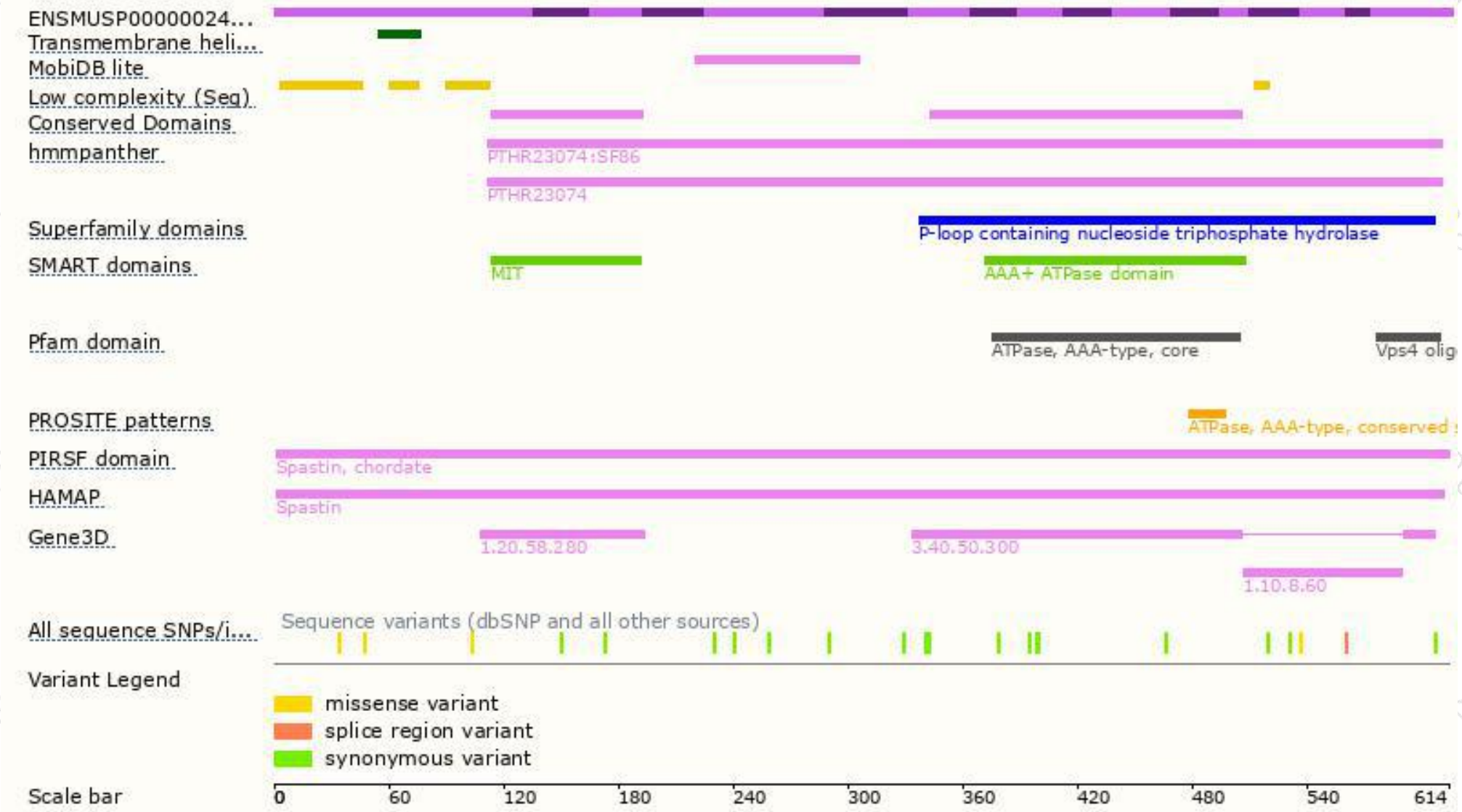
The strategy is based on the design of *Spast-201* transcript,The transcription is shown below



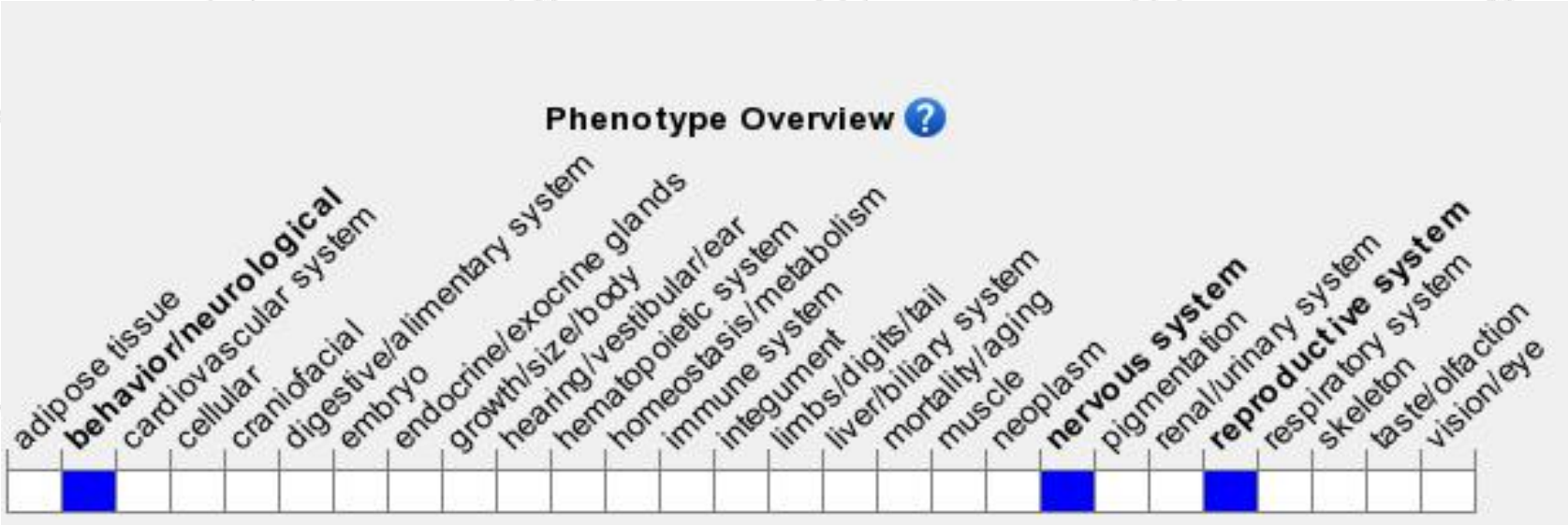
Genomic location distribution



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.

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