Chd8 Cas9-CKO Strategy

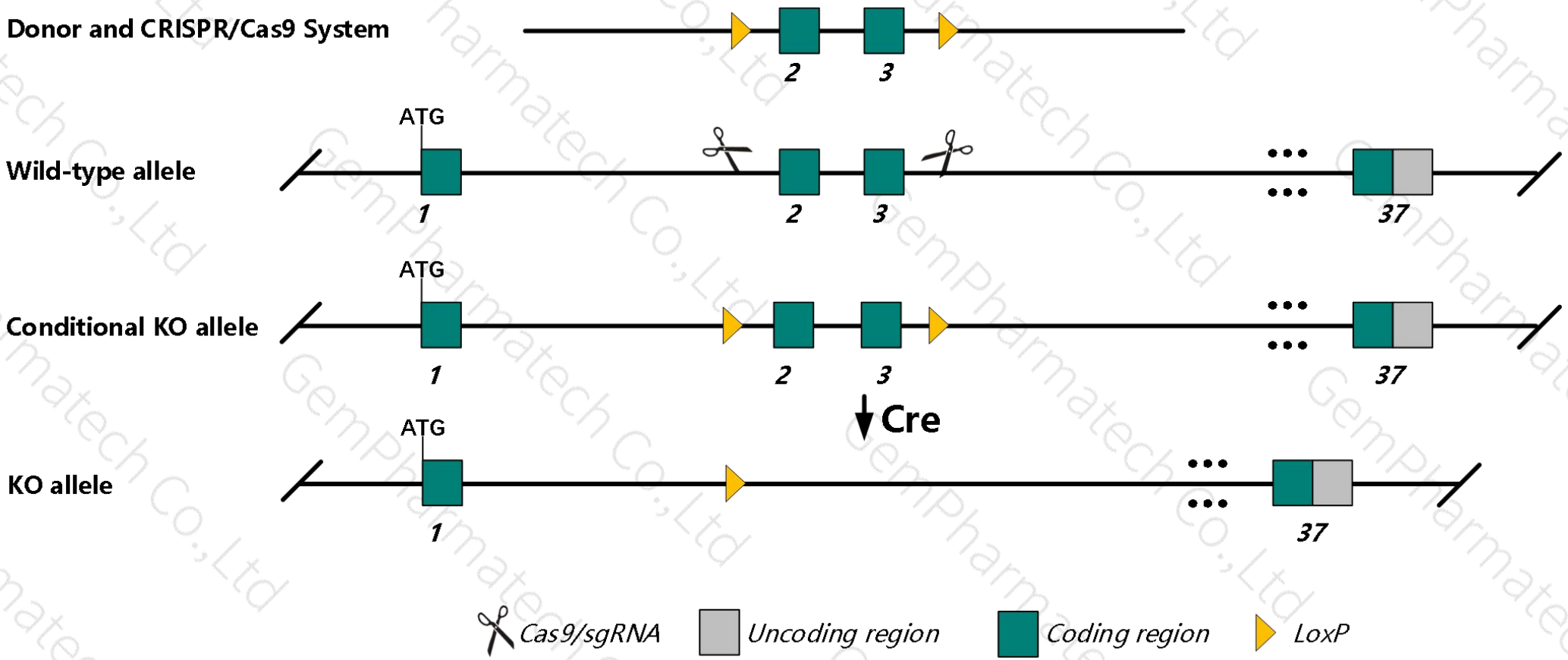
Designer: Lixin Lv

Project Overview

Project Name	***Chd8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Chd8* gene. The schematic diagram is as follows:



Technical routes

- The *Chd8* gene has 16 transcripts. According to the structure of *Chd8* gene, exon2-exon3 of *Chd8-201* (ENSMUST00000089752.10) transcript is recommended as the knockout region.The region contains 764bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Chd8* 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,Homozygous null embryos are growth retarded starting at E5.5 and exhibit developmental arrest at E6.5. Mutants develop into an egg cylinder but do not form a primitive streak or mesoderm and exhibit increased apoptosis at E7.5.

➢ The transcript *Chd8-209*, *Chd8-213* and *Chd8-216* are incomplete, so the effect on them are unknown.

➢ The *Chd8* gene is located on the Chr14. 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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Chd8 chromodomain helicase DNA binding protein 8 [Mus musculus (house mouse)]

Gene ID: 67772, updated on 19-Mar-2019

Summary

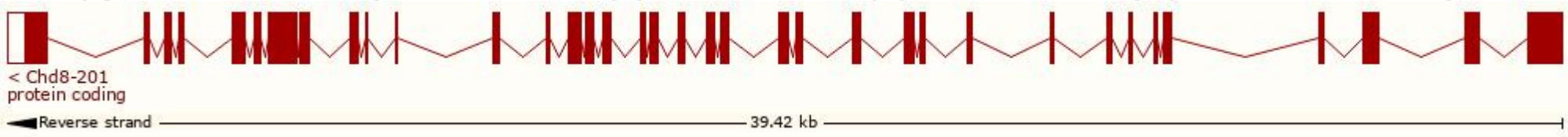
Official Symbol	Chd8 provided by MGI
Official Full Name	chromodomain helicase DNA binding protein 8 provided by MGI
Primary source	MGI:MGI:1915022
See related	Ensembl:ENSMUSG00000053754
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5830451P18Rik, AU015341, Chd-8, Duplin, HELSNF1, mKIAA1564
Summary	This gene encodes a member of the chromodomain-helicase-DNA binding protein family, which is characterized by a SNF2-like domain and two chromatin organization modifier domains. The encoded protein also contains brahma and kismet domains, which is common to the subfamily of chromodomain-helicase-DNA binding proteins to which this protein belongs. In mammals, this gene has been shown to function in several processes including transcriptional regulation, epigenetic remodeling, promotion of cell proliferation, and regulation of RNA synthesis. Knockout of this gene causes early embryonic lethality due to widespread apoptosis. Heterozygous loss of function mutations result in autism spectrum disorder-like behaviors that include increased anxiety, repetitive behavior, and altered social behavior. [provided by RefSeq, Dec 2016]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 11.5), thymus adult (RPKM 10.3) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

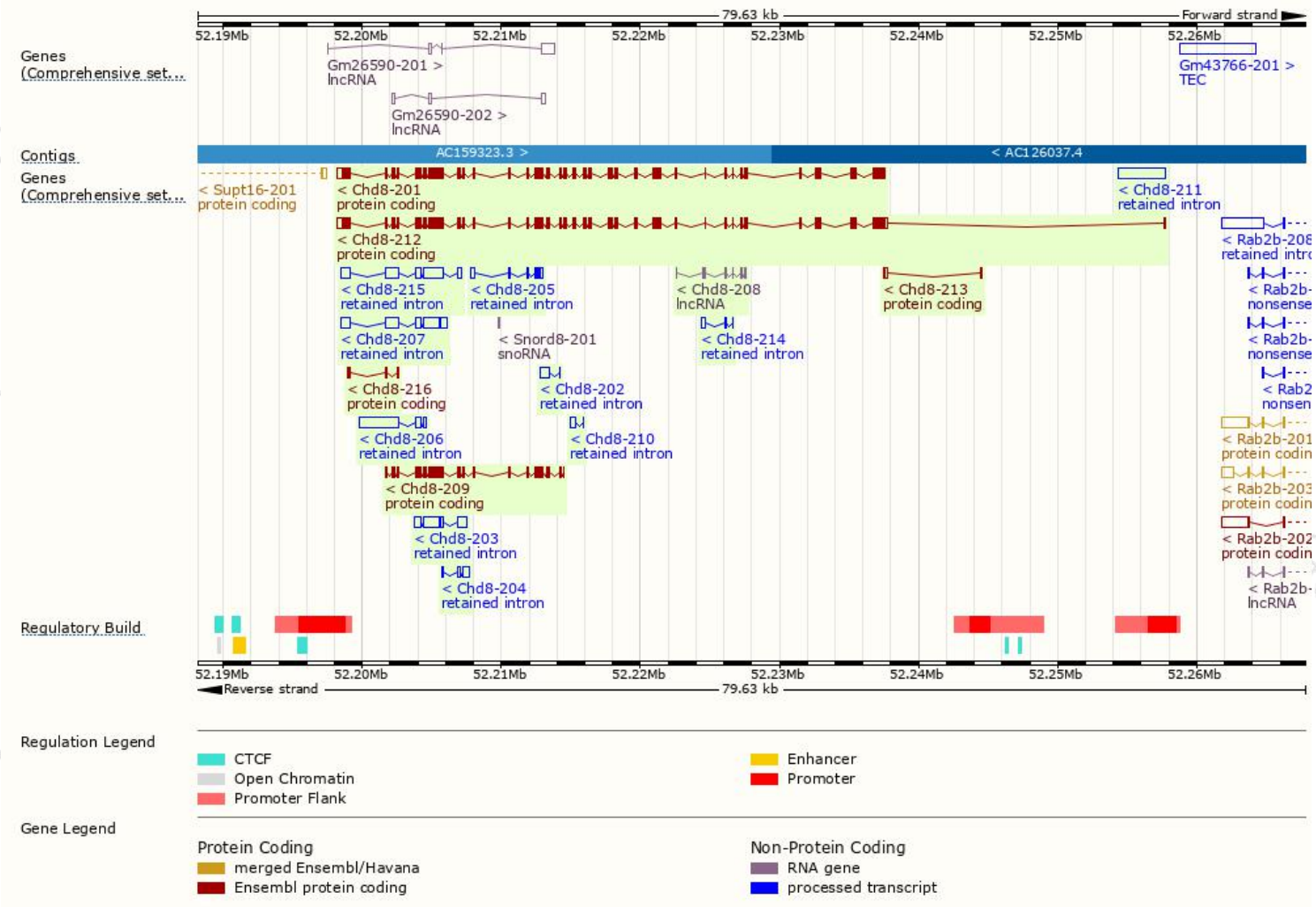
The gene has 16 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Chd8-212	ENSMUST00000200169.5	8509	2582aa	Protein coding	CCDS36919	Q09XV5	TSL:5 GENCODE basic APPRIS P1
Chd8-201	ENSMUST00000089752.10	8190	2582aa	Protein coding	CCDS36919	Q09XV5	TSL:1 GENCODE basic APPRIS P1
Chd8-209	ENSMUST00000149975.8	3094	1031aa	Protein coding	-	F7AL76	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Chd8-216	ENSMUST00000227897.1	415	138aa	Protein coding	-	A0A2I3BRI1	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete
Chd8-213	ENSMUST00000226307.1	360	22aa	Protein coding	-	A0A2I3BR97	CDS 3' incomplete
Chd8-208	ENSMUST00000149694.1	479	No protein	Processed transcript	-	-	TSL:5
Chd8-215	ENSMUST00000226681.1	3695	No protein	Retained intron	-	-	
Chd8-207	ENSMUST00000147827.7	3543	No protein	Retained intron	-	-	TSL:1
Chd8-206	ENSMUST00000147309.1	3350	No protein	Retained intron	-	-	TSL:1
Chd8-211	ENSMUST00000199135.1	3329	No protein	Retained intron	-	-	TSL:NA
Chd8-203	ENSMUST00000134329.1	2444	No protein	Retained intron	-	-	TSL:1
Chd8-205	ENSMUST00000145404.1	911	No protein	Retained intron	-	-	TSL:3
Chd8-204	ENSMUST00000136528.1	741	No protein	Retained intron	-	-	TSL:3
Chd8-202	ENSMUST00000122823.1	674	No protein	Retained intron	-	-	TSL:5
Chd8-214	ENSMUST00000226625.1	488	No protein	Retained intron	-	-	
Chd8-210	ENSMUST00000155614.1	428	No protein	Retained intron	-	-	TSL:3

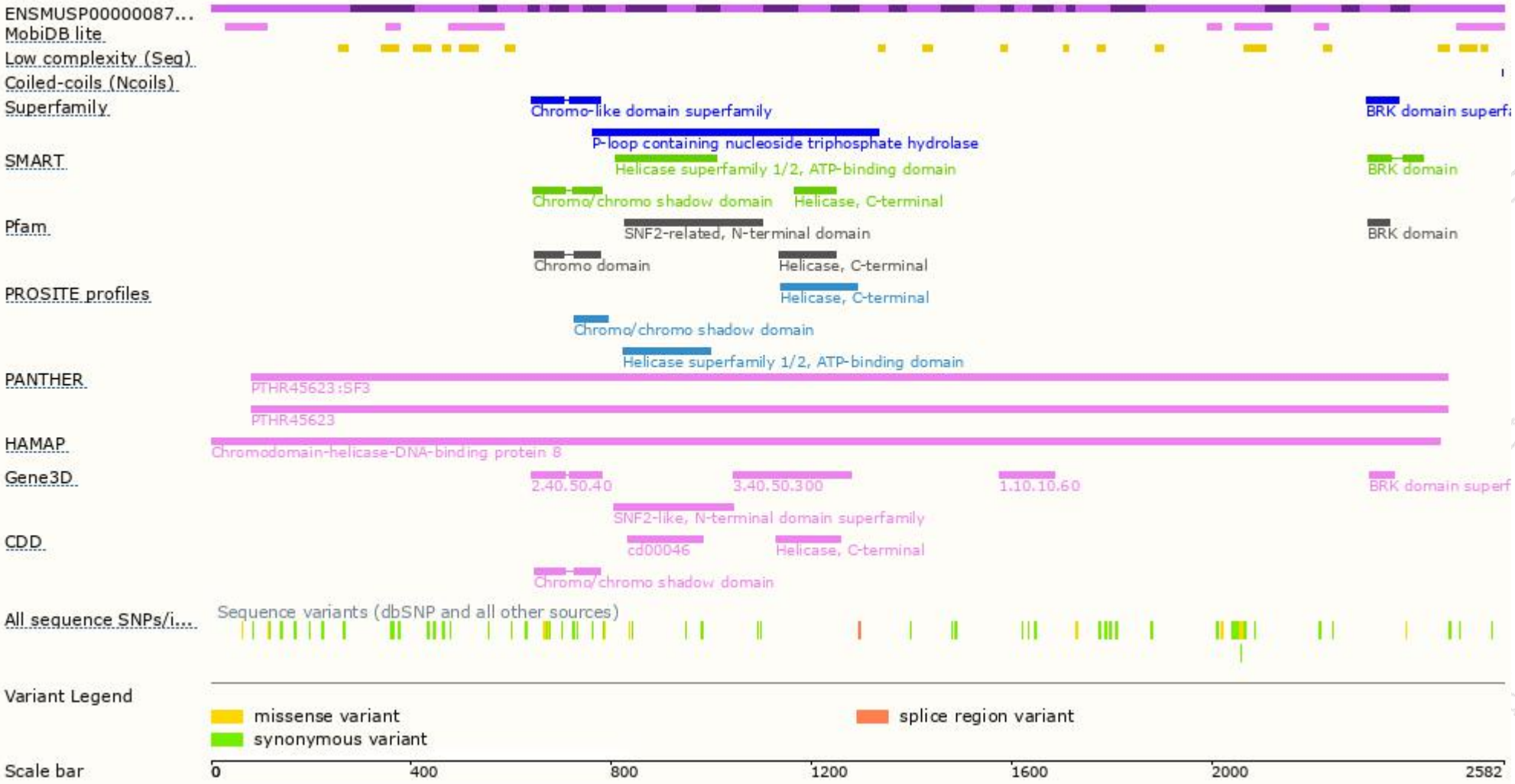
The strategy is based on the design of *Chd8-201* transcript,The transcription is shown below



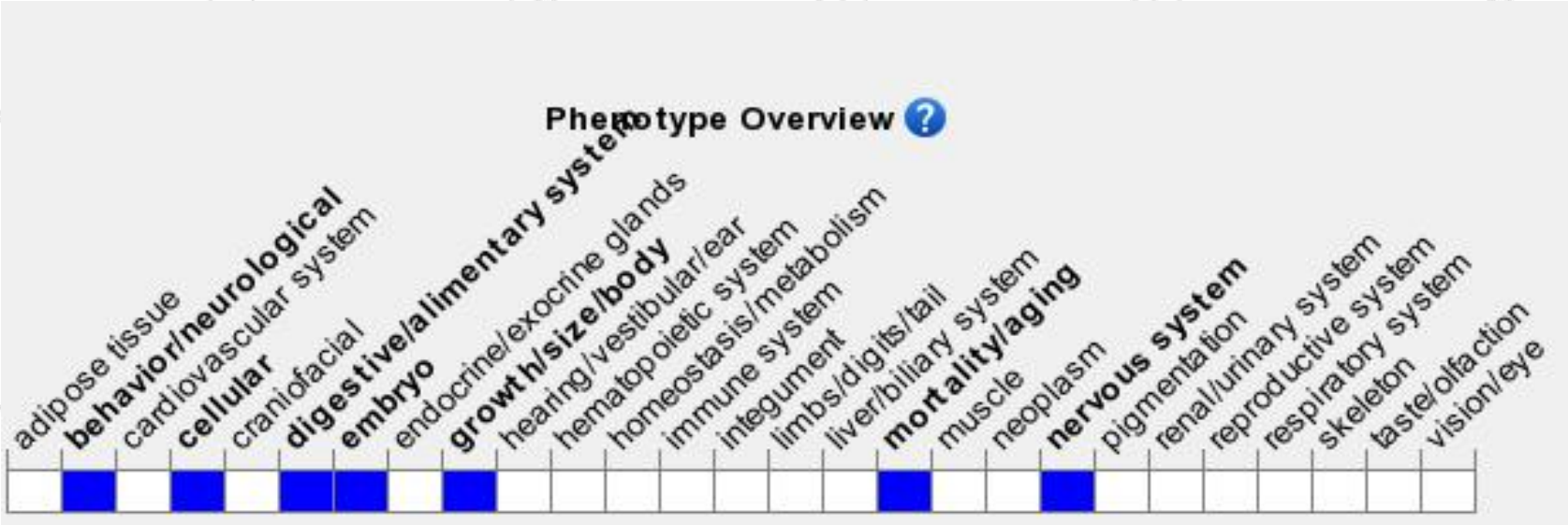
Genomic location distribution



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/).

According to the existing MGI data,Homozygous null embryos are growth retarded starting at E5.5 and exhibit developmental arrest at E6.5. Mutants develop into an egg cylinder but do not form a primitive streak or mesoderm and exhib increased apoptosis at E7.5.

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

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