Nedd4l Cas9-CKO Strategy

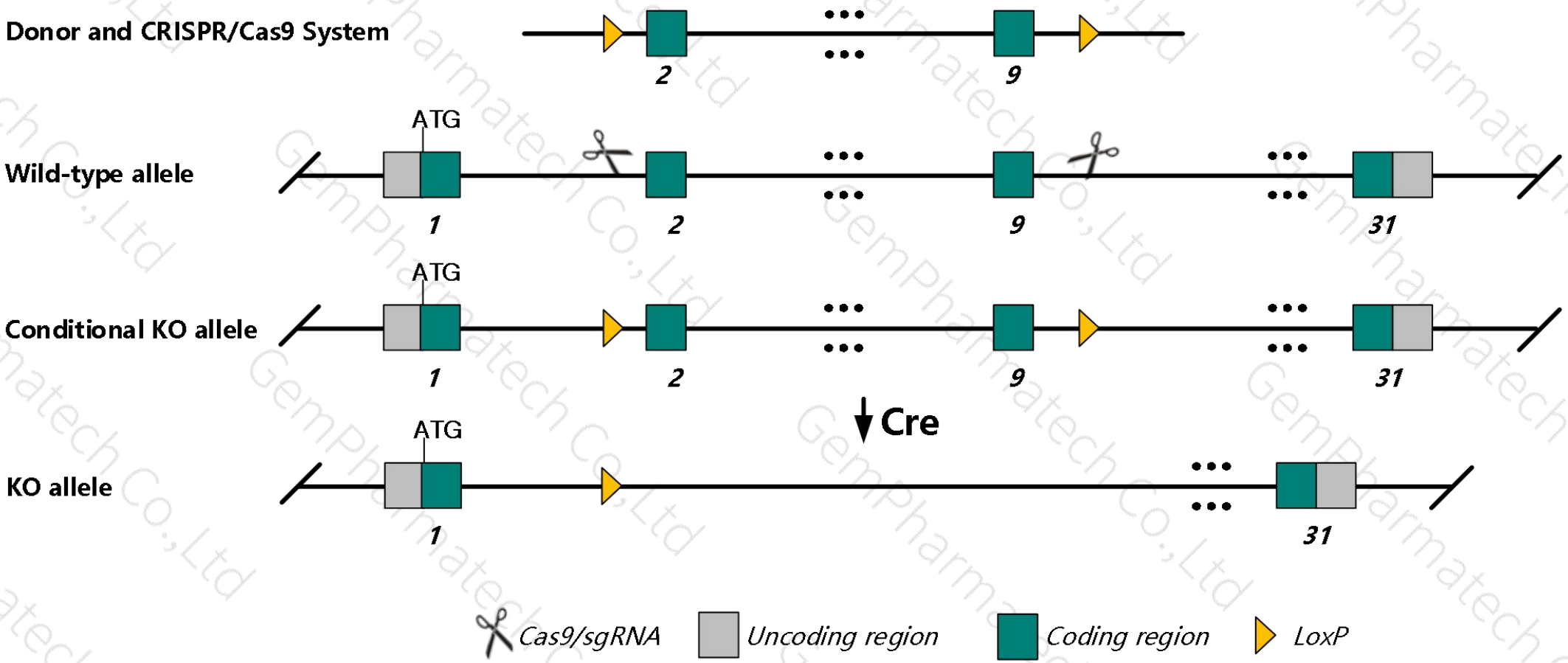
Designer: JiaYu

Project Overview

Project Name	***Nedd4l***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nedd4l* gene. The schematic diagram is as follows:



Technical routes

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- The *Nedd4l* gene has 34 transcripts. According to the structure of *Nedd4l* gene, exon2-exon9 of *Nedd4l-215* (ENSMUST00000235343.1) transcript is recommended as the knockout region.The region contains 632bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nedd4l* 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 salt sensitive hypertension and high salt diet induced cardiac hypertrophy. A spontaneous mutation results in overt diabetes insipidus. Mice homozygous for a knock-out allele exhibit neonatal lethality with primary atelectasis.

➢ The *Nedd4l* gene is located on the Chr18. 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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Nedd4l neural precursor cell expressed, developmentally down-regulated gene 4-like [Mus musculus (house mouse)]

Gene ID: 83814, updated on 12-Mar-2019

Summary

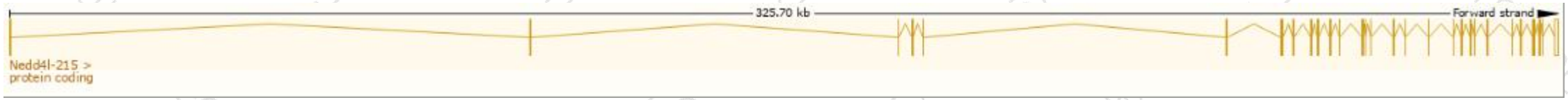
Official Symbol	Nedd4l provided by MGI
Official Full Name	neural precursor cell expressed, developmentally down-regulated gene 4-like provided by MGI
Primary source	MGI:MGI:1933754
See related	Ensembl:ENSMUSG00000024589
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1300012C07Rik, Nedd4-2, Nedd4b
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 4.8), CNS E18 (RPKM 4.7) and 26 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

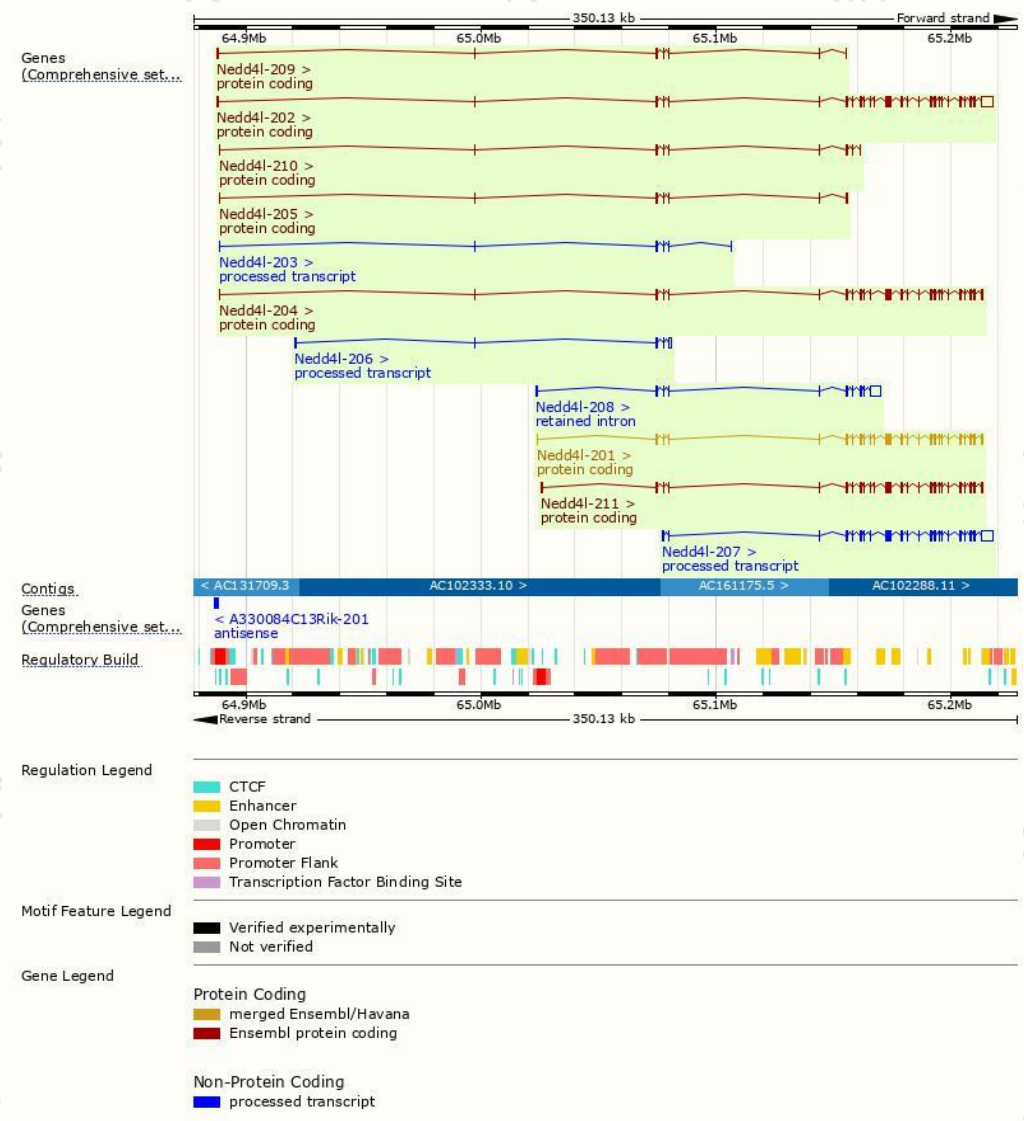
The gene has 34 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nedd4l-215	ENSMUST00000235343.1	3792	976aa	Protein coding	CCDS50309	-	GENCODE basic APPRIS P2
Nedd4l-201	ENSMUST00000080418.6	3671	855aa	Protein coding	CCDS29305	Q3X9H8	TSL:1 GENCODE basic
Nedd4l-228	ENSMUST00000237384.1	3592	855aa	Protein coding	CCDS29305	-	GENCODE basic
Nedd4l-211	ENSMUST00000226058.1	3393	855aa	Protein coding	CCDS29305	Q3X9H8	GENCODE basic
Nedd4l-204	ENSMUST00000224347.2	3295	855aa	Protein coding	CCDS29305	A0A286YCM8	GENCODE basic
Nedd4l-202	ENSMUST00000163516.8	7787	835aa	Protein coding	-	E9PXB7	TSL:5 GENCODE basic
Nedd4l-233	ENSMUST00000237854.1	4845	835aa	Protein coding	-	-	GENCODE basic
Nedd4l-223	ENSMUST00000236736.1	4802	956aa	Protein coding	-	-	GENCODE basic APPRIS ALT2
Nedd4l-221	ENSMUST00000236209.1	3254	912aa	Protein coding	-	-	GENCODE basic
Nedd4l-220	ENSMUST00000236103.1	3182	751aa	Protein coding	-	-	GENCODE basic
Nedd4l-225	ENSMUST00000236898.1	3034	1004aa	Protein coding	-	-	GENCODE basic
Nedd4l-229	ENSMUST00000237410.1	2974	984aa	Protein coding	-	-	GENCODE basic
Nedd4l-218	ENSMUST00000235743.1	2917	872aa	Protein coding	-	-	GENCODE basic
Nedd4l-231	ENSMUST00000237644.1	2018	527aa	Protein coding	-	-	CDS 5' incomplete
Nedd4l-217	ENSMUST00000235577.1	770	256aa	Protein coding	-	-	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete
Nedd4l-210	ENSMUST00000225261.2	747	224aa	Protein coding	-	A0A286YCR1	CDS 3' incomplete
Nedd4l-205	ENSMUST00000224385.2	735	113aa	Protein coding	-	A0A286YDV4	CDS 3' incomplete
Nedd4l-213	ENSMUST00000235310.1	586	94aa	Protein coding	-	-	CDS 3' incomplete
Nedd4l-224	ENSMUST00000236764.1	548	37aa	Protein coding	-	-	CDS 3' incomplete
Nedd4l-207	ENSMUST00000224663.2	4875	No protein	Processed transcript	-	-	
Nedd4l-226	ENSMUST00000236916.1	792	No protein	Processed transcript	-	-	
Nedd4l-212	ENSMUST00000235204.1	776	No protein	Processed transcript	-	-	
Nedd4l-222	ENSMUST00000236595.1	717	No protein	Processed transcript	-	-	
Nedd4l-203	ENSMUST00000223959.1	414	No protein	Processed transcript	-	-	
Nedd4l-209	ENSMUST00000225057.2	381	No protein	Processed transcript	-	-	
Nedd4l-234	ENSMUST00000238122.1	373	No protein	Processed transcript	-	-	
Nedd4l-219	ENSMUST00000236100.1	348	No protein	Processed transcript	-	-	
Nedd4l-227	ENSMUST00000236929.1	240	No protein	Processed transcript	-	-	
Nedd4l-208	ENSMUST00000224890.1	5814	No protein	Retained intron	-	-	
Nedd4l-216	ENSMUST00000235571.1	1116	No protein	Retained intron	-	-	
Nedd4l-206	ENSMUST00000224516.1	1045	No protein	Retained intron	-	-	
Nedd4l-214	ENSMUST00000235339.1	772	No protein	Retained intron	-	-	
Nedd4l-232	ENSMUST00000237818.1	550	No protein	Retained intron	-	-	
Nedd4l-230	ENSMUST00000237492.1	437	No protein	Retained intron	-	-	

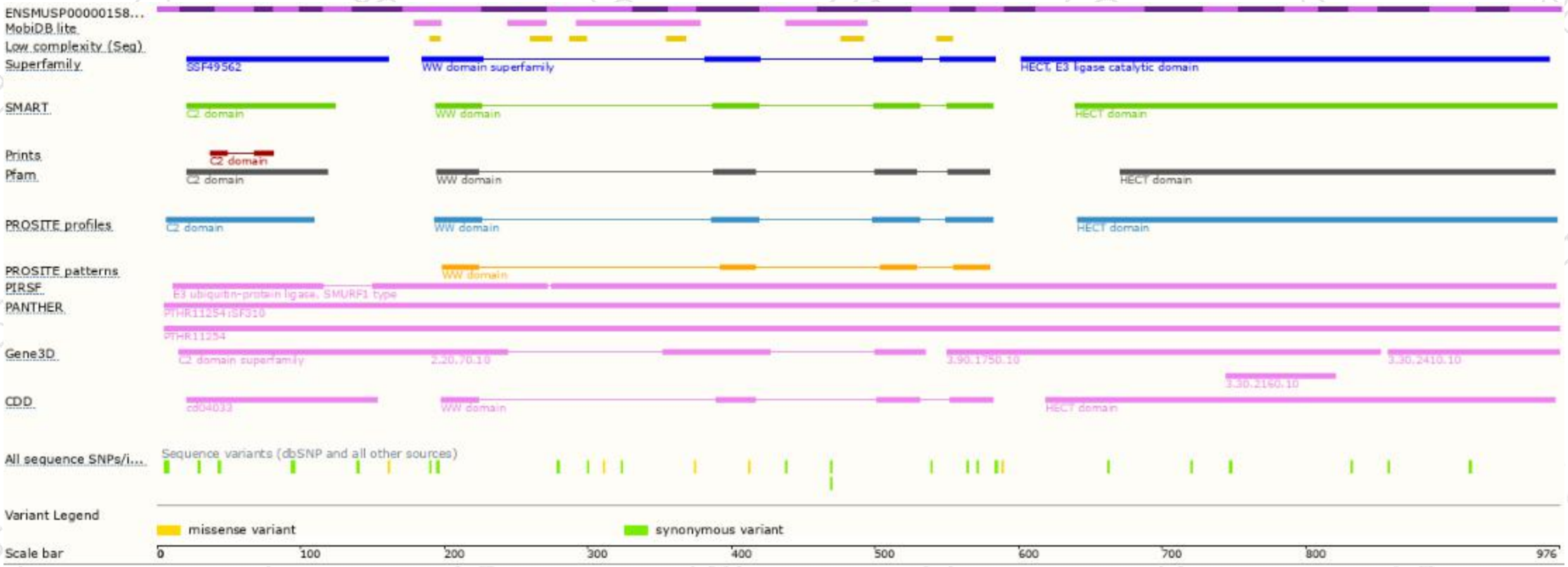
The strategy is based on the design of *Nedd4l-215* transcript,The transcription is shown below



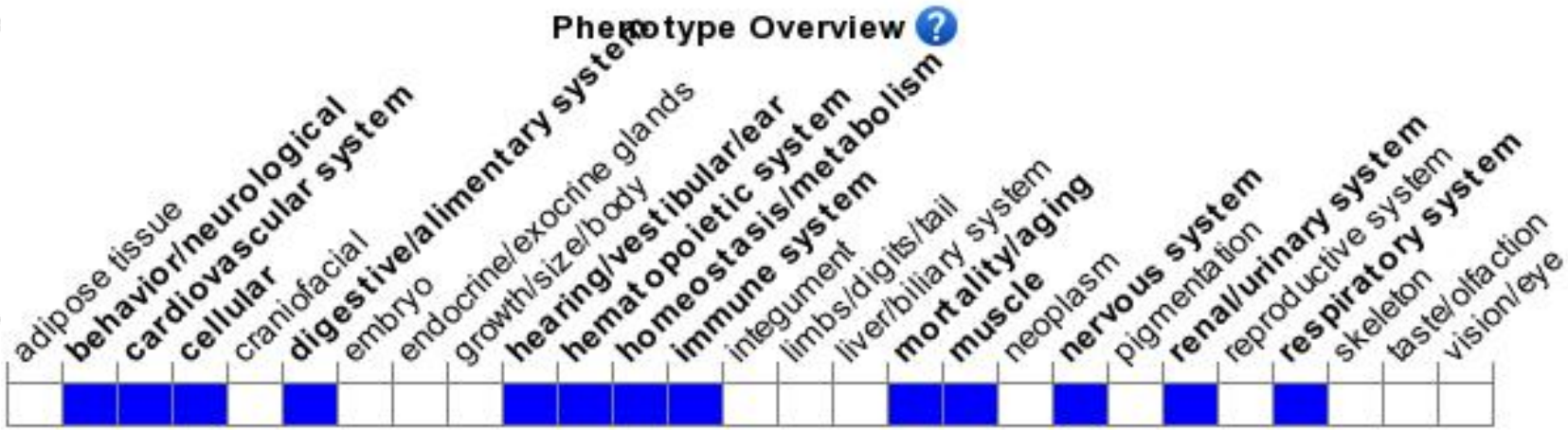
Genomic location distribution



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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