Depdc5 Cas9-CKO Strategy

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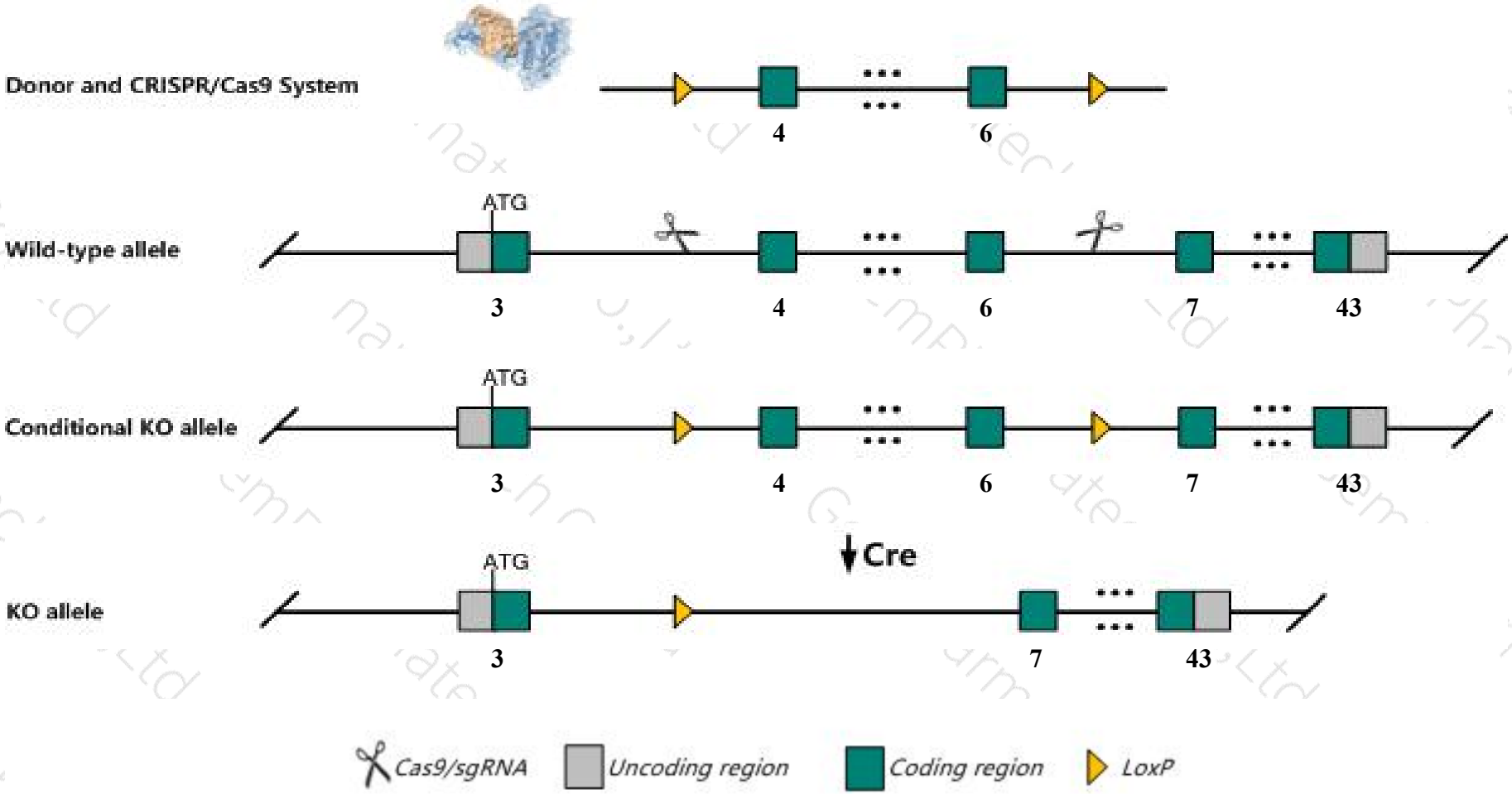
Project Overview

Project Name	***Depdc5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Depdc5* gene. The schematic diagram is as follows:



Technical routes

➢ The *Depdc5* gene has 20 transcripts. According to the structure of *Depdc5* gene, exon4-exon6 of *Depdc5-202*(ENSMUST00000087897.10) transcript is recommended as the knockout region.The region contains 221bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Depdc5* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 knock-out allele exhibit preweaning lethality. Mice homozygous for a conditional allele activated in neurons exhibit reduced body weight, limb grasping, premature death, spontaneous seizure, increased brain size due to neuron hypertrophy and increased PTZ seizure susceptibility.

➢ Transcript *Depdc5-209 and Depdc5-210* may not be affected.

➢ The *Depdc5* gene is located on the Chr5. 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）

集萃药康 GemPharmatech

Depdc5 DEP domain containing 5 [Mus musculus (house mouse)]

Gene ID: 277854, updated on 13-Mar-2020

Summary

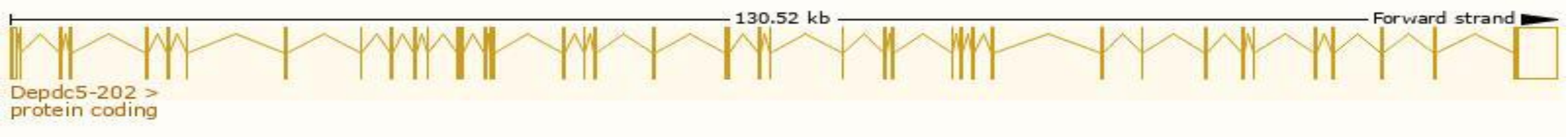
Official Symbol	Depdc5 provided by MGI
Official Full Name	DEP domain containing 5 provided byMGI
Primary source	MGI:MGI:2141101
See related	Ensembl:ENSMUSG00000037426
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AV016528
Expression	Ubiquitous expression in cerebellum adult (RPKM 5.6), whole brain E14.5 (RPKM 5.6) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

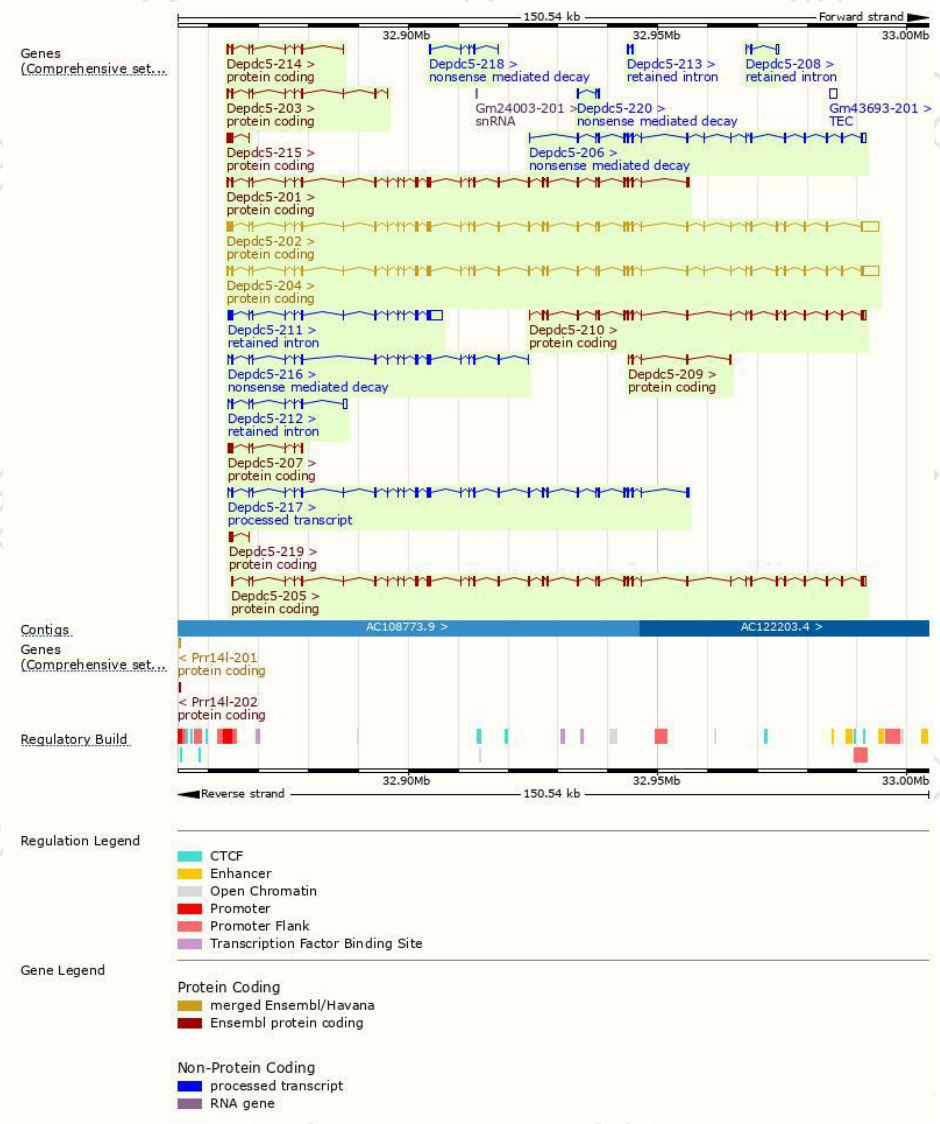
The gene has 20 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Depdc5-202	ENSMUST00000087897.10	8216	1530aa	Protein coding	CCDS19196	Q6GQV2	TSL:1 GENCODE basic
Depdc5-204	ENSMUST00000119705.7	7929	1591aa	Protein coding	CCDS51463	P61460	TSL:1 GENCODE basic APPRIS P2
Depdc5-201	ENSMUST00000049780.12	3569	1085aa	Protein coding	CCDS19197	E9QAT3	TSL:1 GENCODE basic
Depdc5-205	ENSMUST00000120902.7	5195	1569aa	Protein coding	-	P61460	TSL:5 GENCODE basic APPRIS ALT1
Depdc5-210	ENSMUST00000137169.7	3359	976aa	Protein coding	-	F6TK47	CDS 5' incomplete TSL:5
Depdc5-203	ENSMUST00000118698.7	720	205aa	Protein coding	-	E9PWF2	CDS 3' incomplete TSL:5
Depdc5-207	ENSMUST00000125574.7	683	137aa	Protein coding	-	D3Z7M7	CDS 3' incomplete TSL:3
Depdc5-209	ENSMUST00000130461.1	620	206aa	Protein coding	-	F6XIK0	CDS 5' and 3' incomplete TSL:5
Depdc5-214	ENSMUST00000149350.7	585	161aa	Protein coding	-	D3Z5Y4	CDS 3' incomplete TSL:5
Depdc5-215	ENSMUST00000150130.7	346	31aa	Protein coding	-	D3Z419	CDS 3' incomplete TSL:2
Depdc5-219	ENSMUST00000202927.1	327	35aa	Protein coding	-	A0A0J9YV31	CDS 3' incomplete TSL:3
Depdc5-206	ENSMUST00000124780.7	3176	657aa	Nonsense mediated decay	-	F6X8H7	CDS 5' incomplete TSL:5
Depdc5-216	ENSMUST00000195980.4	1732	153aa	Nonsense mediated decay	-	A0A0G2JFM3	TSL:1
Depdc5-218	ENSMUST00000201836.1	428	44aa	Nonsense mediated decay	-	A0A0J9YU49	CDS 5' incomplete TSL:3
Depdc5-220	ENSMUST00000238200.1	175	26aa	Nonsense mediated decay	-	-	CDS 5' incomplete
Depdc5-217	ENSMUST00000201802.3	3511	No protein	Processed transcript	-	-	TSL:1
Depdc5-211	ENSMUST00000139098.7	3872	No protein	Retained intron	-	-	TSL:2
Depdc5-212	ENSMUST00000139463.5	1219	No protein	Retained intron	-	-	TSL:1
Depdc5-208	ENSMUST00000127560.1	583	No protein	Retained intron	-	-	TSL:3
Depdc5-213	ENSMUST00000141812.1	431	No protein	Retained intron	-	-	TSL:2

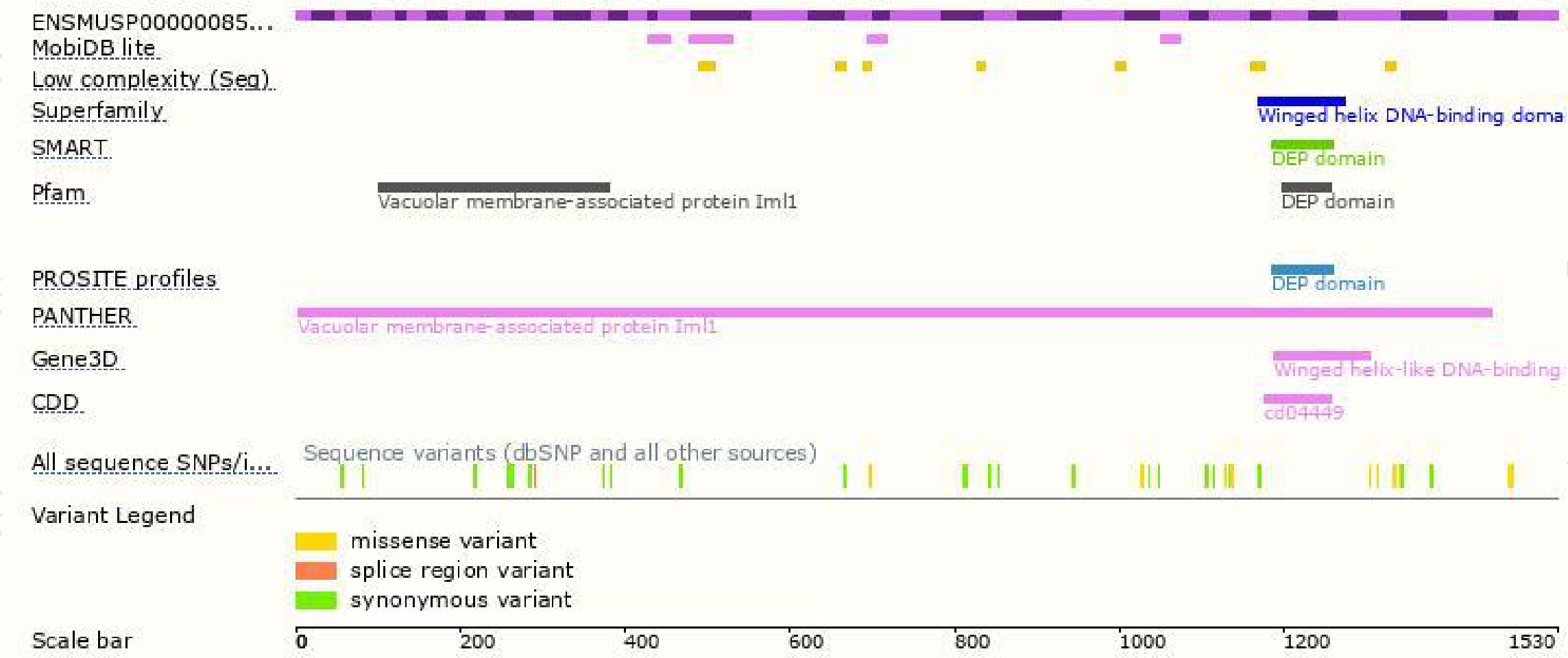
The strategy is based on the design of *Depdc5-202* transcript,the transcription is shown below:



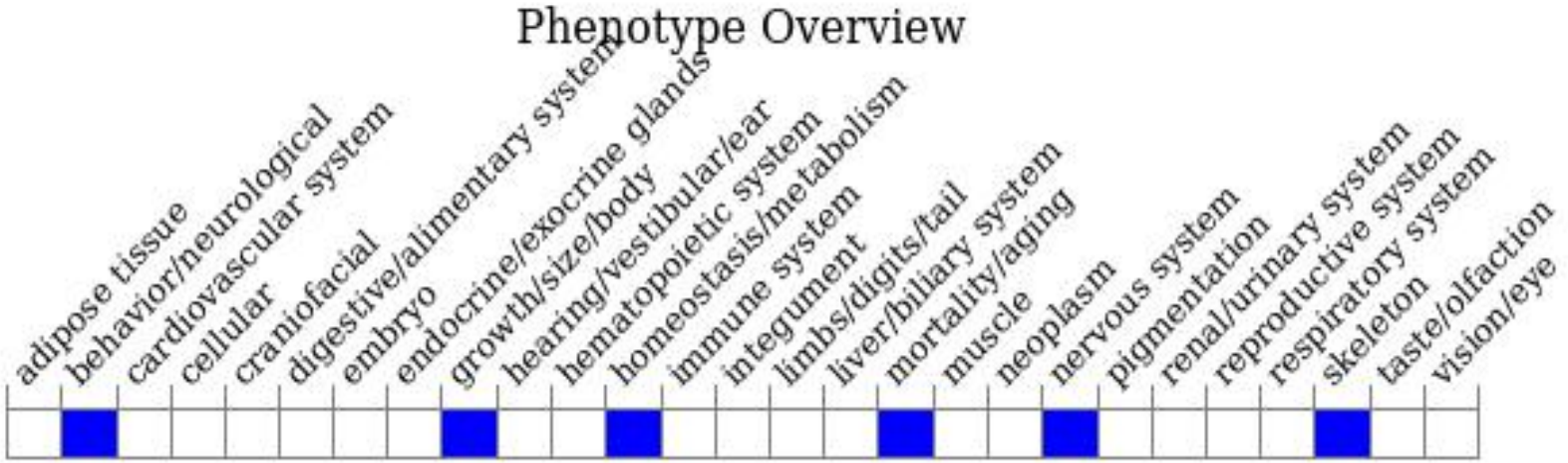
Genomic location distribution



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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