Car8 Cas9-CKO Strategy

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Project Overview

Project Name	***Car8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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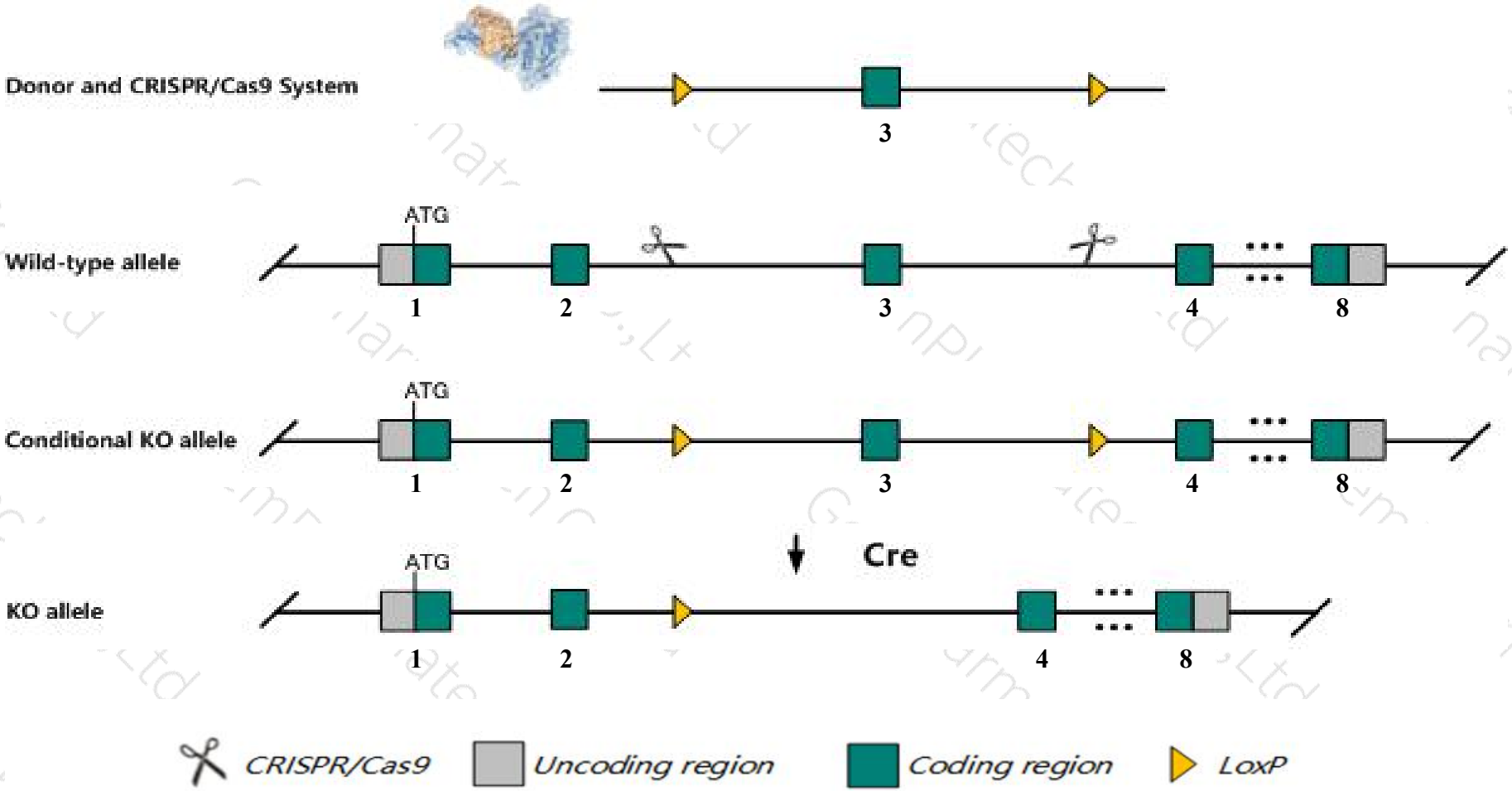
This model will use CRISPR/Cas9 technology to edit the *Car8* gene. The schematic diagram is as follows:



Technical routes

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- The *Car8* gene has 1 transcript. According to the structure of *Car8* gene, exon3 of *Car8-201* (ENSMUST00000066674.7) transcript is recommended as the knockout region.The region contains 125bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Car8* 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 spontaneous deletion exhibit a wobbly side-to-side gait which is first noted at two weeks of age and persists throughout life.

- The N-terminal of *Car8* gene will remain several amino acids ,it may remain the partial function of *Car8* gene.

- The *Car8* gene is located on the Chr4. 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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Car8 carbonic anhydrase 8 [*Mus musculus* (house mouse)]

Gene ID: 12319, updated on 17-Dec-2019

Summary

Official Symbol	Car8 provided by MGI
Official Full Name	carbonic anhydrase 8 provided by MGI
Primary source	MGI:MGI:88253
See related	Ensembl:ENSMUSG00000041261
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	Ca8; wdl; Cals; Carp; Cals1; AW546993
Expression	Biased expression in cerebellum adult (RPKM 76.1) and lung adult (RPKM 3.9) See more
Orthologs	human all

Genomic context

Location: 4 A1; 4 3.53 cM

See Car8 in Genome Data Viewer

Exon count: 9

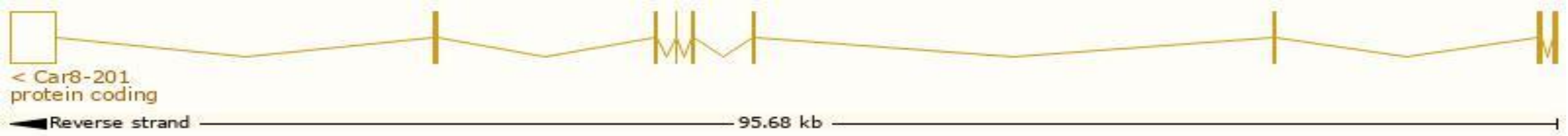
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	4	NC_000070.6 (8141493..8239041, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	4	NC_000070.5 (8068640..8166188, complement)

Transcript information（Ensembl）

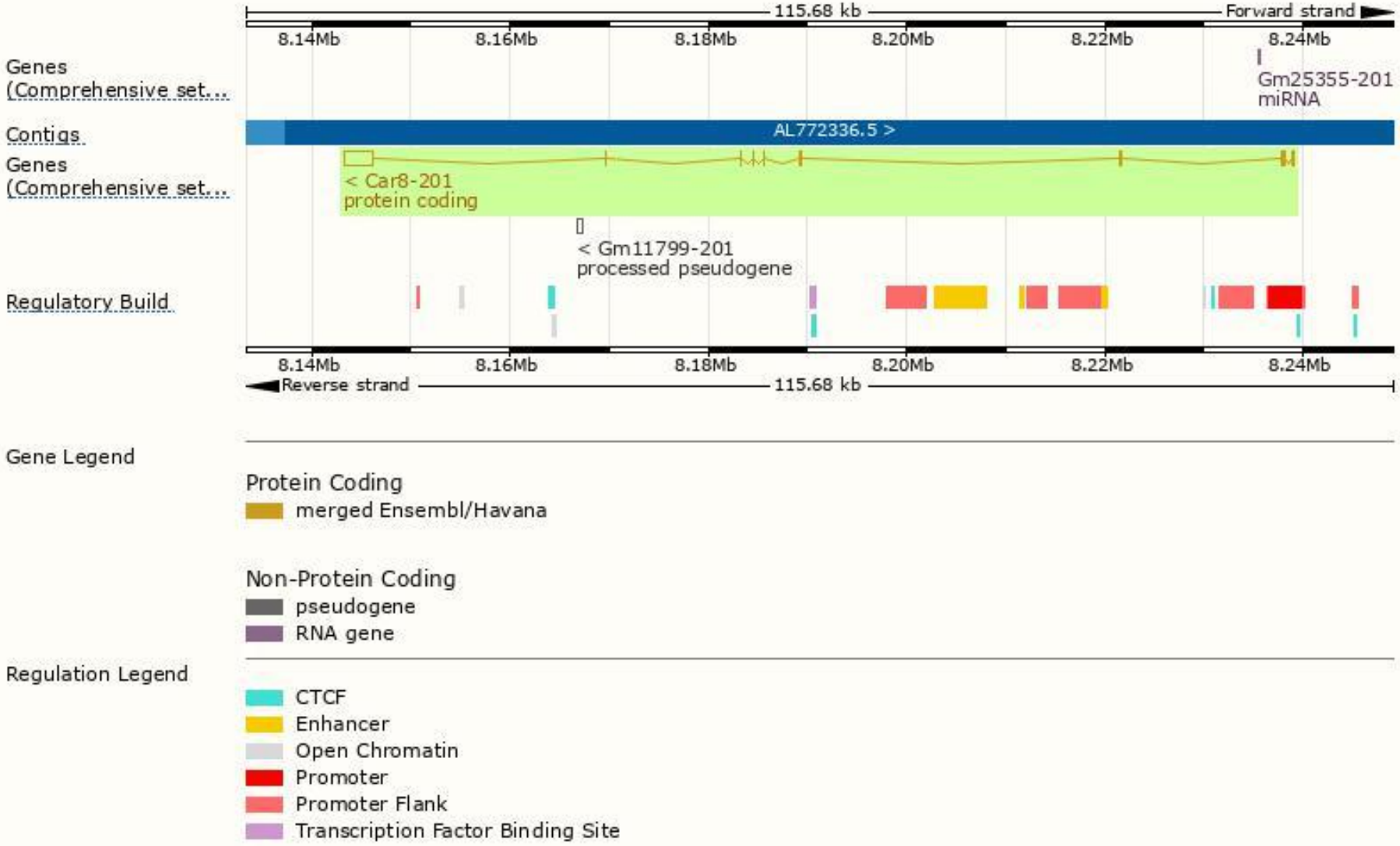
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Car8-201	ENSMUST00000066674.7	3853	291aa	Protein coding	CCDS17954	P28651	TSL:1 GENCODE basic APPRIS P1

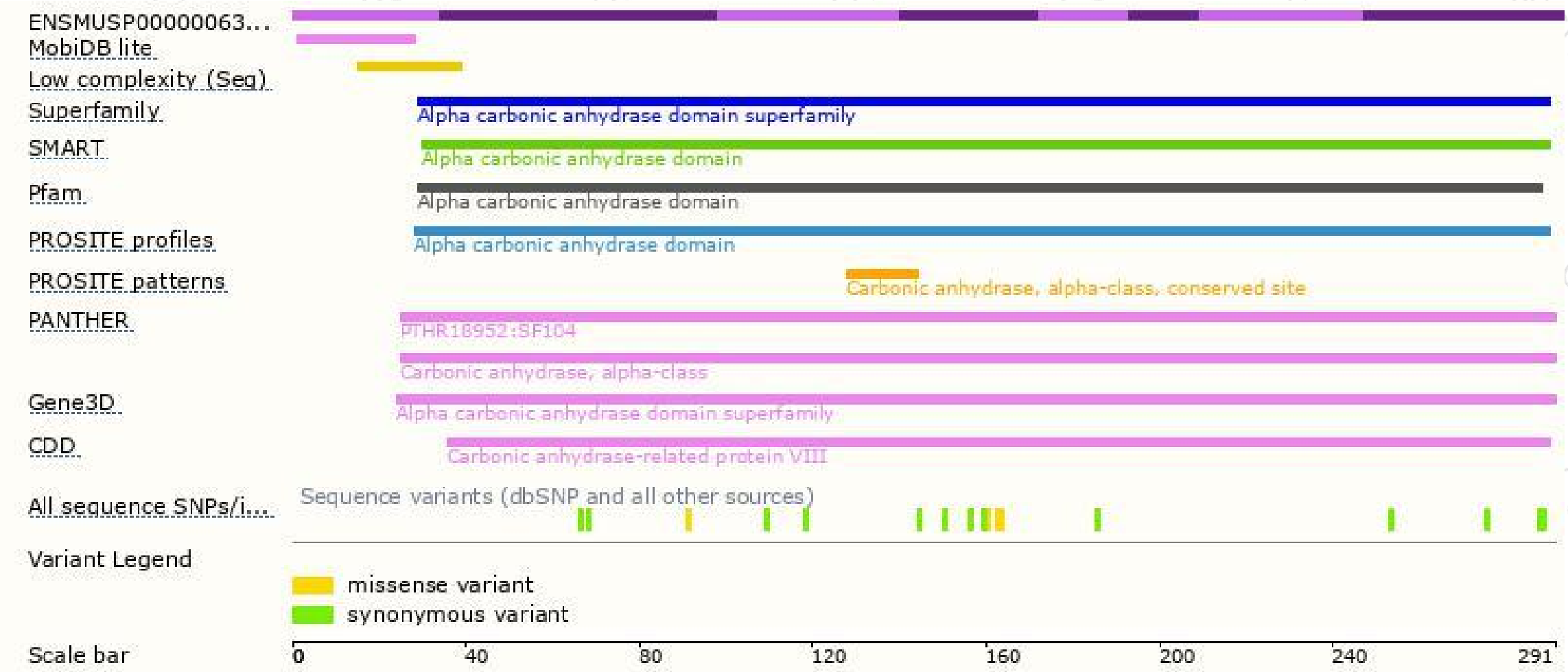
The strategy is based on the design of *Car8-201* transcript,The transcription is shown below



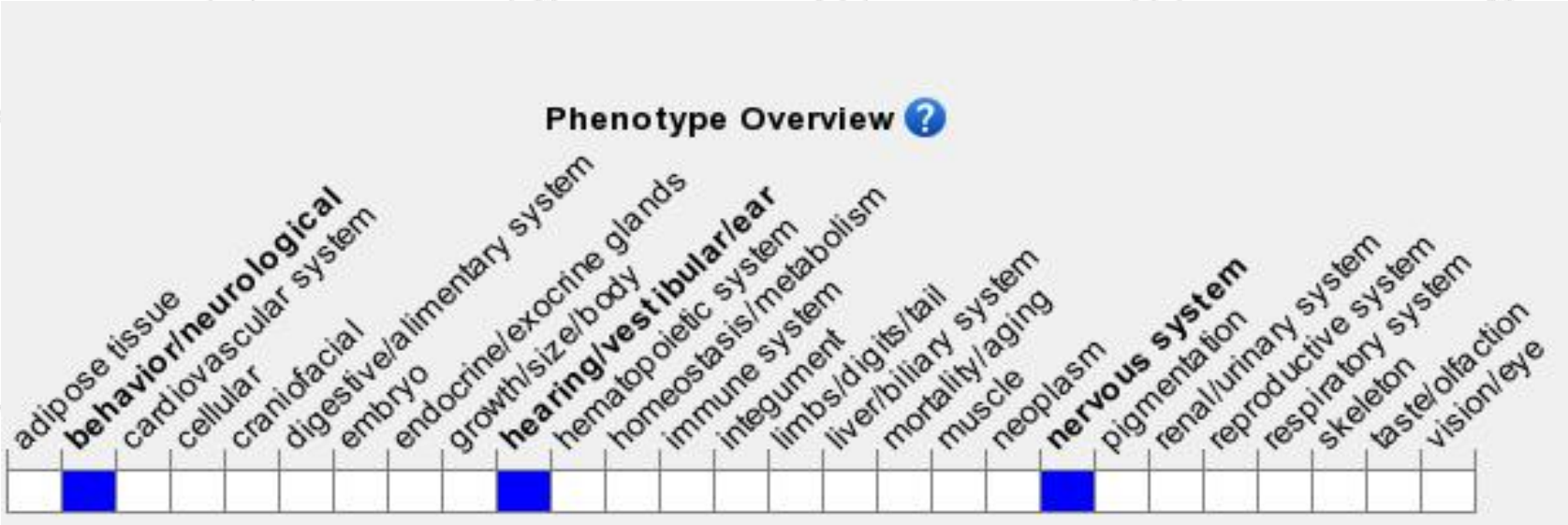
Genomic location distribution



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,Mice homozygous for a spontaneous deletion exhibit a wobbly side-to-side gait which is first noted at two weeks of age and persists throughout life.

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

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