Nectin3 Cas9-KO Strategy

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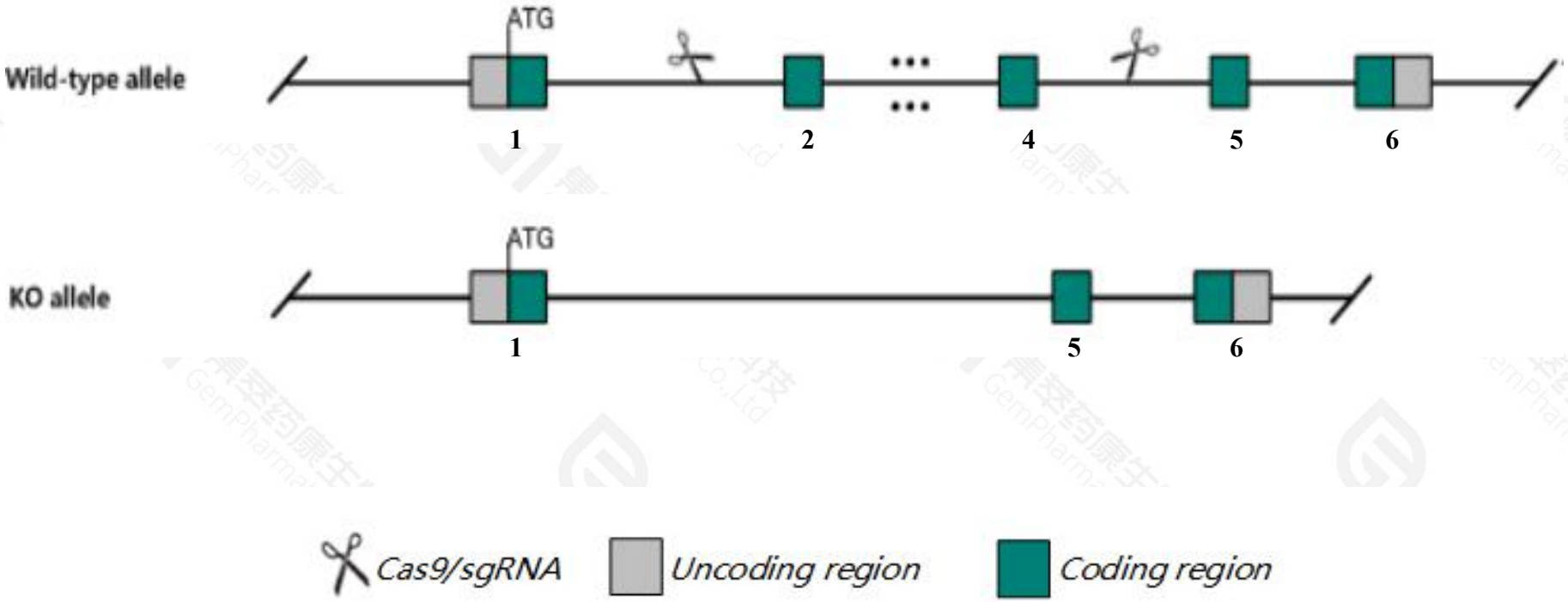
Project Overview

Project Name	***Nectin3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nectin3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Nectin3* gene has 10 transcripts. According to the structure of *Nectin3* gene, exon2-exon4 of *Nectin3-201*(ENSMUST00000023334.15) transcript is recommended as the knockout region.The region contains 757bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Nectin3* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data,homozygous null mice exhibit male infertility and eye abnormalities including microphthalmia, absent vitreous body, abnormal ciliary body, retinal layers, and lenses.

➢ The *Nectin3* gene is located on the Chr16. 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.

➢Transcript 204.205.206. 207.208 will not be destroyed.

➢ This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Nectin3 nectin cell adhesion molecule 3 [Mus musculus (house mouse)]

Gene ID: 58998, updated on 21-Feb-2021

Summary

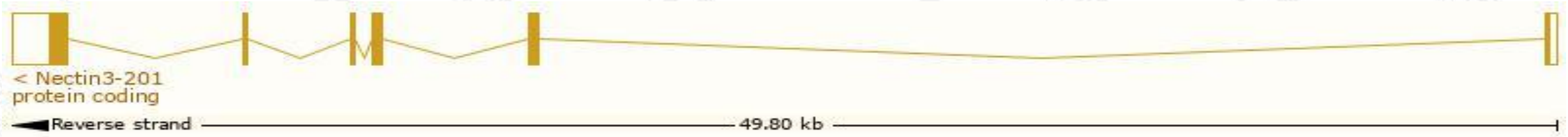
Official Symbol	Nectin3 provided by MGI
Official Full Name	nectin cell adhesion molecule 3 provided by MGI
Primary source	MGI:MGI:1930171
See related	Ensembl:ENSMUSG00000022656
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2610301B19Rik, 3000002N23Rik, 4921513D19Rik, AA407785, AU016832, AW538082, CD113, P, Pvrl3, necti
Expression	Broad expression in testis adult (RPKM 13.1), CNS E11.5 (RPKM 4.7) and 19 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

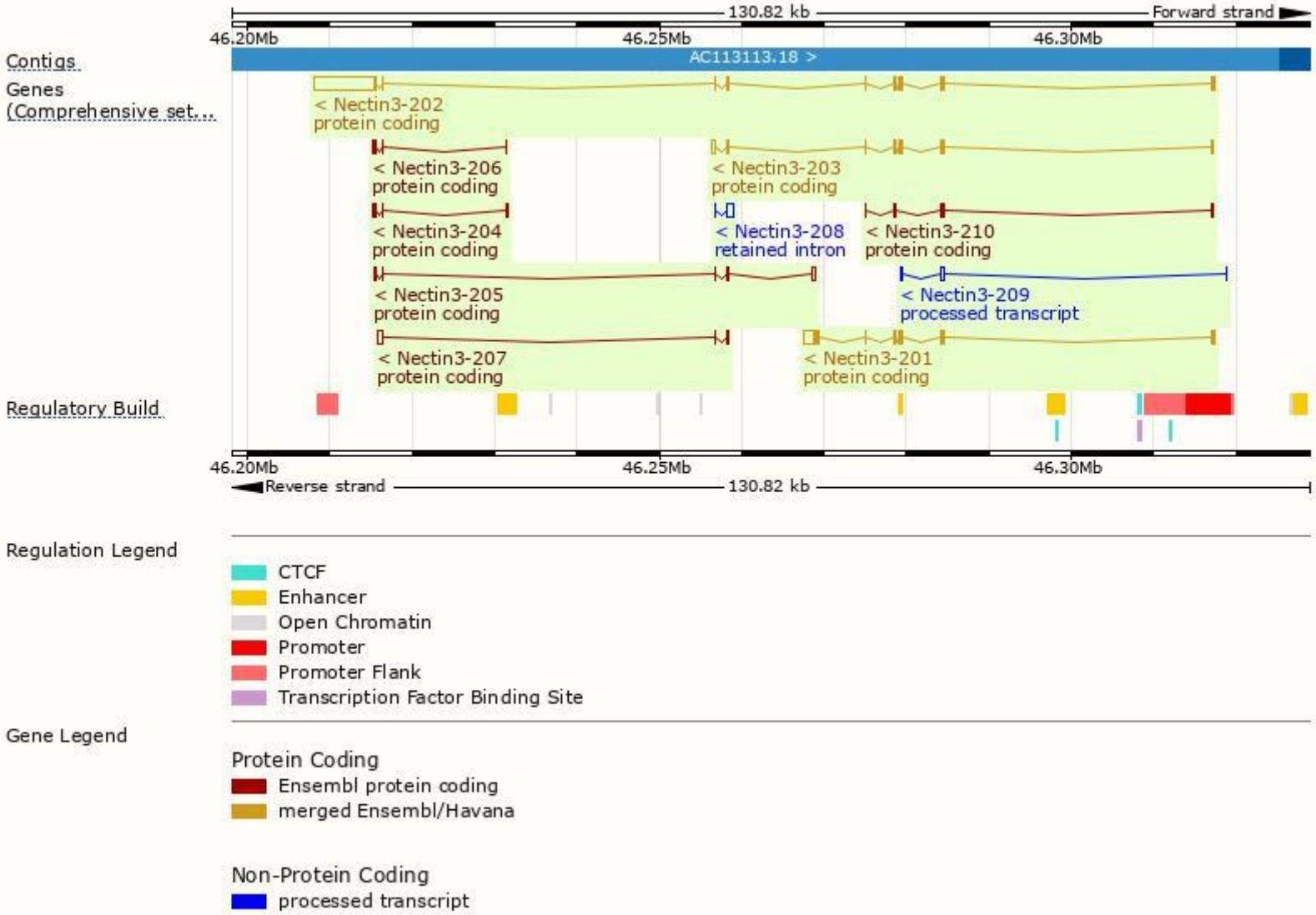
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nectin3-202	ENSMUST00000023335.13	9116	510aa	Protein coding	CCDS28204		TSL:1 , GENCODE basic , APPRIS ALT1 ,
Nectin3-201	ENSMUST00000023334.15	3062	549aa	Protein coding	CCDS28206		TSL:1 , GENCODE basic , APPRIS P4 ,
Nectin3-203	ENSMUST00000096052.9	1746	438aa	Protein coding	CCDS28205		TSL:1 , GENCODE basic ,
Nectin3-205	ENSMUST00000121245.8	953	185aa	Protein coding	-		TSL:3 , GENCODE basic ,
Nectin3-207	ENSMUST00000124602.2	911	122aa	Protein coding	-		CDS 5' incomplete , TSL:3 ,
Nectin3-210	ENSMUST00000149901.2	732	244aa	Protein coding	-		CDS 5' and 3' incomplete , TSL:3 ,
Nectin3-206	ENSMUST00000121803.8	541	100aa	Protein coding	-		TSL:2 , GENCODE basic ,
Nectin3-204	ENSMUST00000119941.2	529	93aa	Protein coding	-		TSL:3 , GENCODE basic ,
Nectin3-209	ENSMUST00000133935.2	737	No protein	Processed transcript	-		TSL:3 ,
Nectin3-208	ENSMUST00000132089.2	752	No protein	Retained intron	-		TSL:2 ,

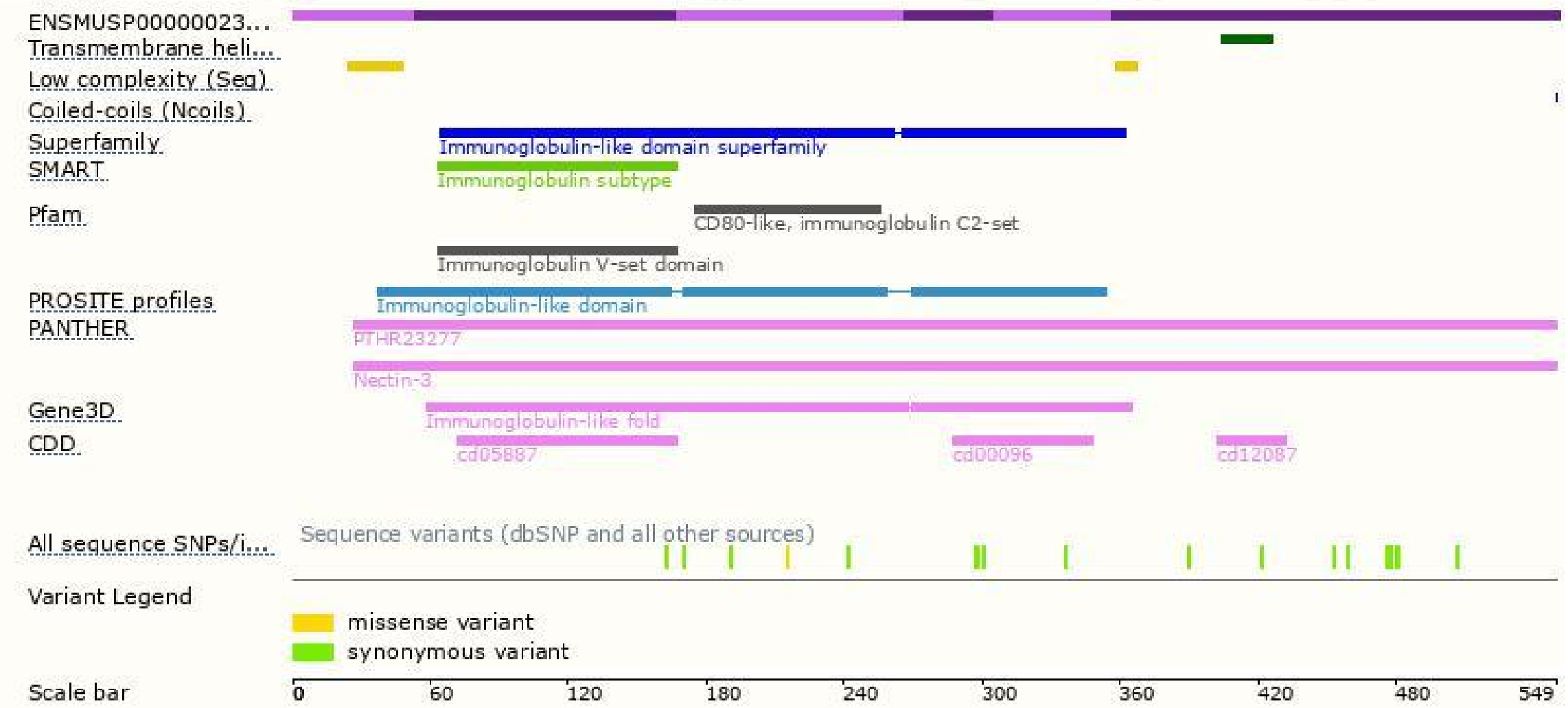
The strategy is based on the design of *Nectin3-201* transcript,the transcription is shown below:



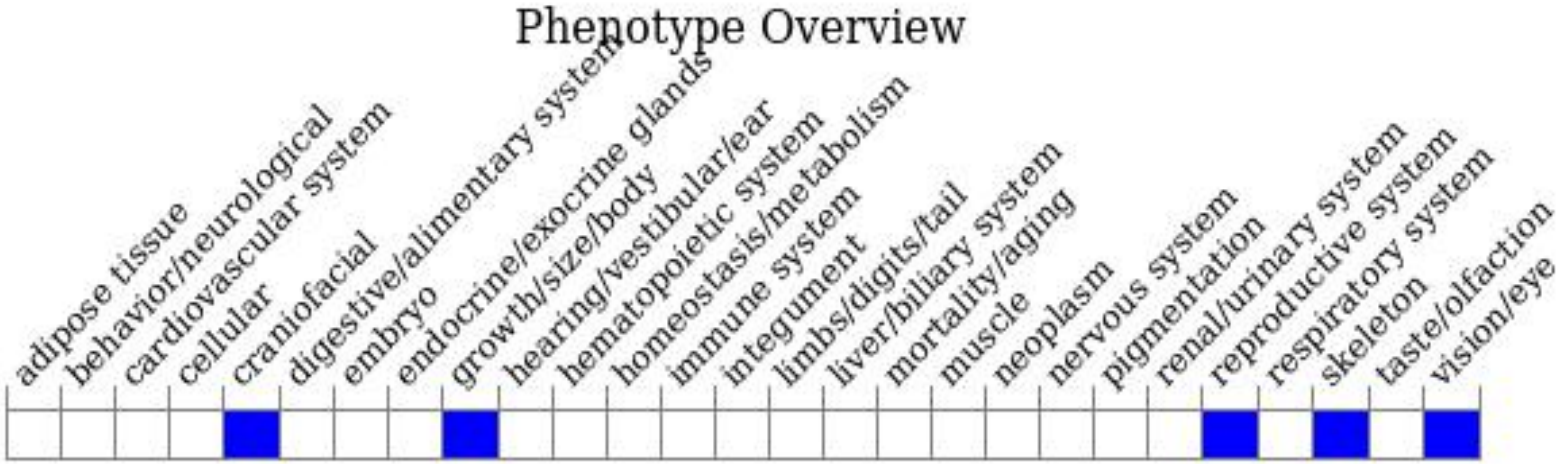
Genomic location distribution



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 mice exhibit male infertility and eye abnormalities including microphthalmia, absent vitreous body, abnormal ciliary body, retinal layers, and lenses.

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

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