Foxn3 Cas9-KO Strategy

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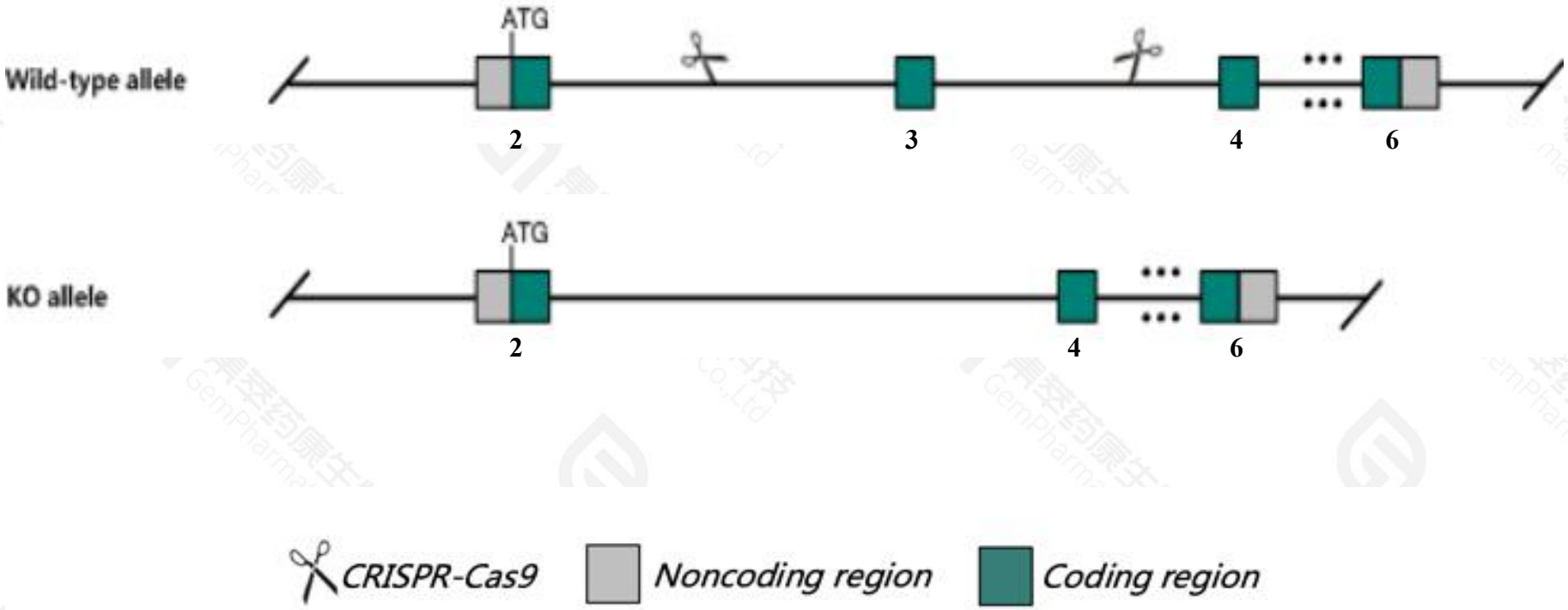
Design Date: 2022-6-6

Project Overview

Project Name	***Foxn3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Foxn3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Foxn3* gene has 14 transcripts. According to the structure of *Foxn3* gene, exon3 of *Foxn3-201*(ENSMUST00000046859.12) transcript is recommended as the knockout region.The region contains 113bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR-Cas9 technology to modify *Foxn3* gene. The brief process is as follows: CRISPR-Cas9 system 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,hypomorphic homozygous knockout affects the expression of osteogenic genes and leads to craniofacial abnormalities and reduces pre- and postnatal survival.

➢ The KO region contains functional region of the Foxn3 gene.Knockout the region may affect the function of 3300002A11Rik gene.

➢ The effect on transcripts Foxn3-205 and Foxn3-214 is unknown.

➢ The N-terminal of Foxn3 gene will remain several amino acids, it may remain the partial function of Foxn3 gene.

➢ The *Foxn3* gene is located on the Chr12. 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 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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Foxn3 forkhead box N3 [*Mus musculus* (house mouse)]

Gene ID: 71375, updated on 24-Apr-2022

Summary

Official Symbol	Foxn3 provided by MGI
Official Full Name	forkhead box N3 provided by MGI
Primary source	MGI:MGI:1918625
See related	Ensembl:ENSMUSG00000033713 AllianceGenome:MGI:1918625
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	Ches1; Ches1l; HTLFL1; AA589593; AW556347; 5430426H20Rik
Summary	Predicted to enable DNA-binding transcription factor activity; cis-regulatory region sequence-specific DNA binding activity; and protein C-terminus binding activity. Acts upstream of or within craniofacial suture morphogenesis. Predicted to be active in nucleus. Is expressed in several structures, including central nervous system; genitourinary system; immune system; peripheral nervous system; and sensory organ. Orthologous to human FOXN3 (forkhead box N3). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in cerebellum adult (RPKM 10.2), bladder adult (RPKM 8.0) and 28 other tissues See more
Orthologs	human all

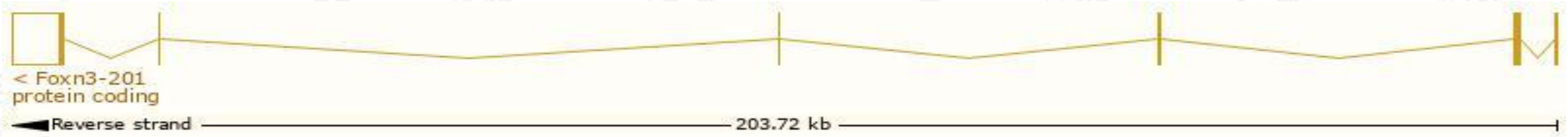
Transcript information（Ensembl）

The gene has 14 transcripts,all transcripts are shown below:

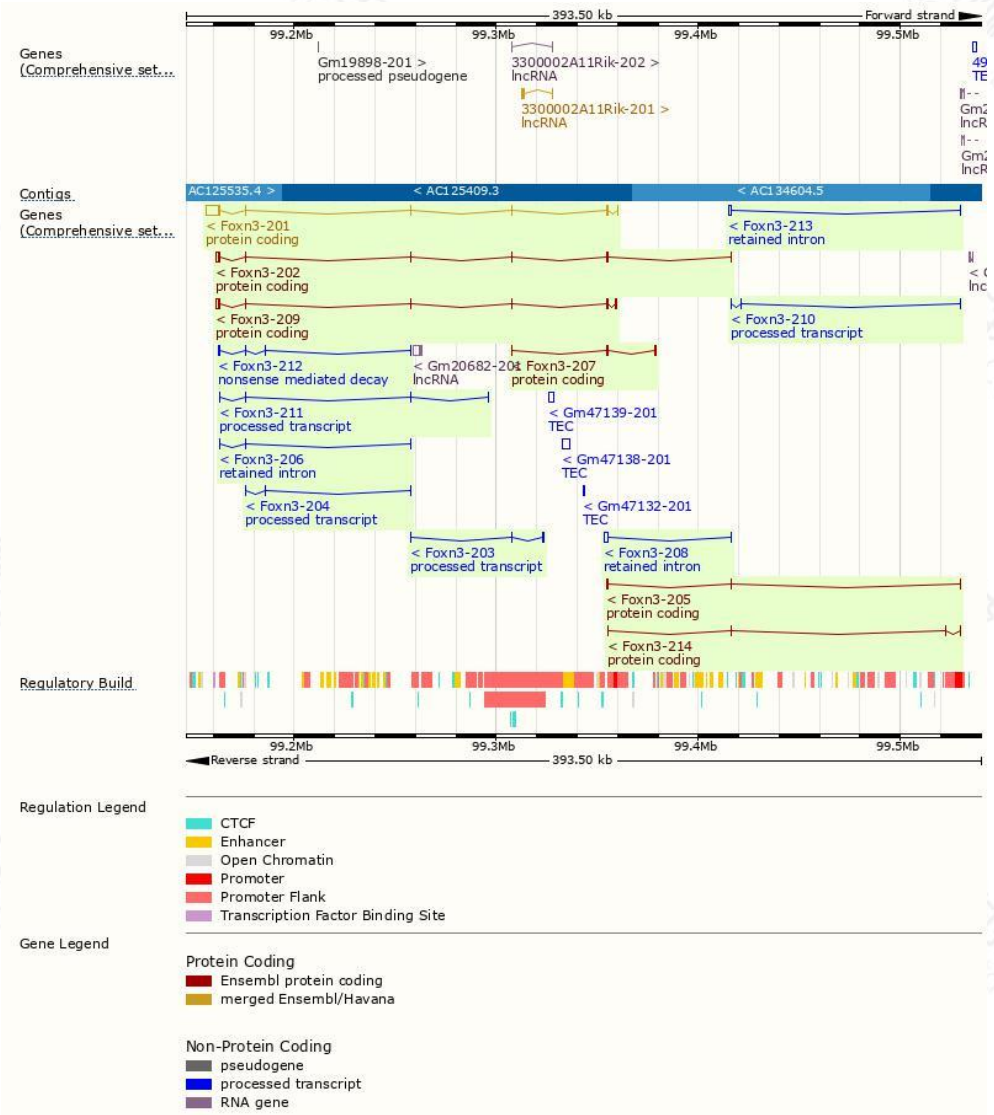
Show/hide columns (1 hidden) Filter

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000046859.12	Foxn3-201	7705	457aa	Protein coding	CCDS26103	Q499D0	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000177451.8	Foxn3-209	2882	457aa	Protein coding	CCDS26103	Q499D0	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000085108.8	Foxn3-202	2611	457aa	Protein coding	CCDS26103	Q499D0	GENCODE basic APPRIS P1 TSL:5
ENSMUST00000177269.2	Foxn3-207	726	191aa	Protein coding		H3BLJ5	TSL:5 CDS 3' incomplete
ENSMUST00000176928.3	Foxn3-205	696	186aa	Protein coding		H3BLD8	TSL:2 CDS 3' incomplete
ENSMUST00000223484.2	Foxn3-214	405	1aa	Protein coding		-	TSL:5 CDS 3' incomplete
ENSMUST00000222458.2	Foxn3-212	617	23aa	Nonsense mediated decay		A0A1Y7VMU1	TSL:5 CDS 5' incomplete
ENSMUST00000176311.2	Foxn3-203	432	No protein	Processed transcript		-	TSL:3
ENSMUST00000222261.2	Foxn3-211	404	No protein	Processed transcript		-	TSL:3
ENSMUST00000221283.2	Foxn3-210	360	No protein	Processed transcript		-	TSL:2
ENSMUST00000176829.2	Foxn3-204	168	No protein	Processed transcript		-	TSL:1
ENSMUST00000177287.2	Foxn3-208	1586	No protein	Retained intron		-	TSL:1
ENSMUST00000222918.2	Foxn3-213	1450	No protein	Retained intron		-	TSL:1
ENSMUST00000176959.3	Foxn3-206	387	No protein	Retained intron		-	TSL:2

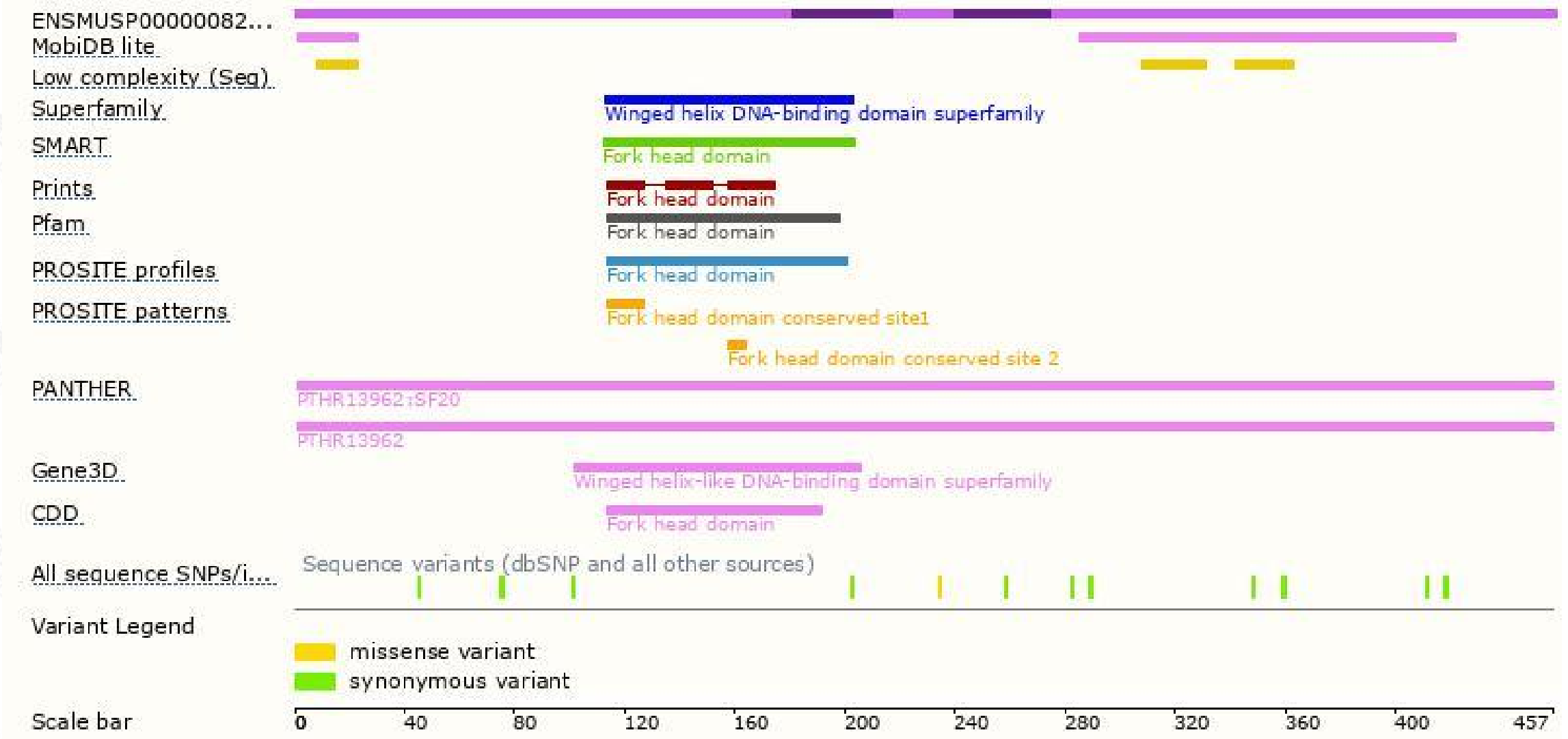
The strategy is based on the design of *Foxn3-201* transcript,the transcription is shown below:



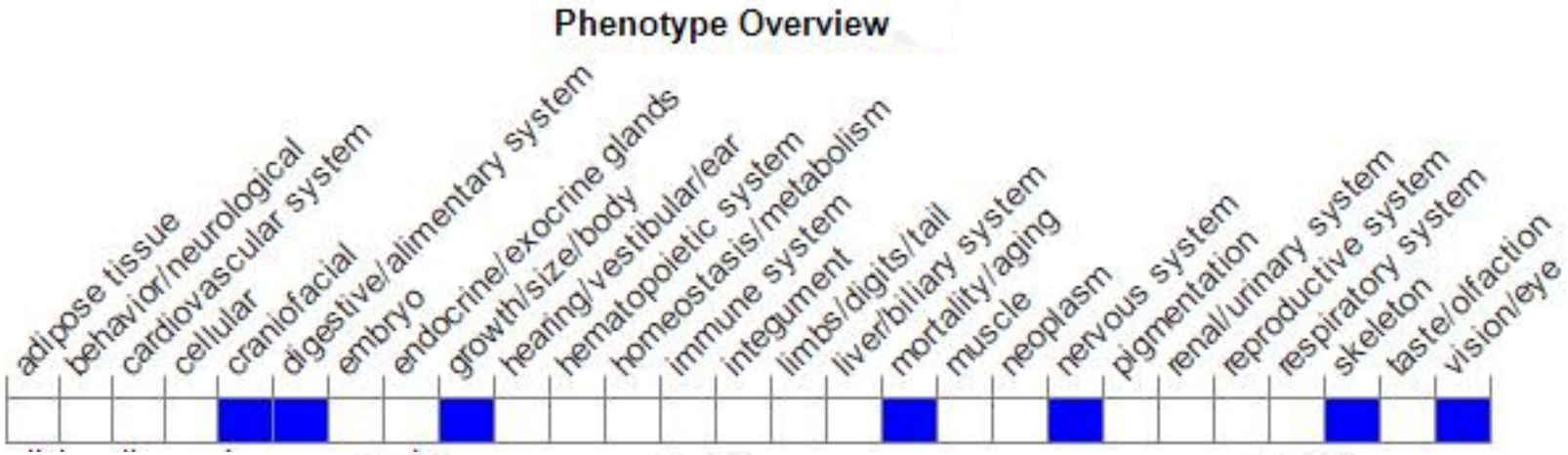
Genomic location distribution



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/).

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