Sema4a Cas9-KO Strategy

Designer:Xueting Zhang
Reviewer:Yanhua Shen
Date:2020-1-19

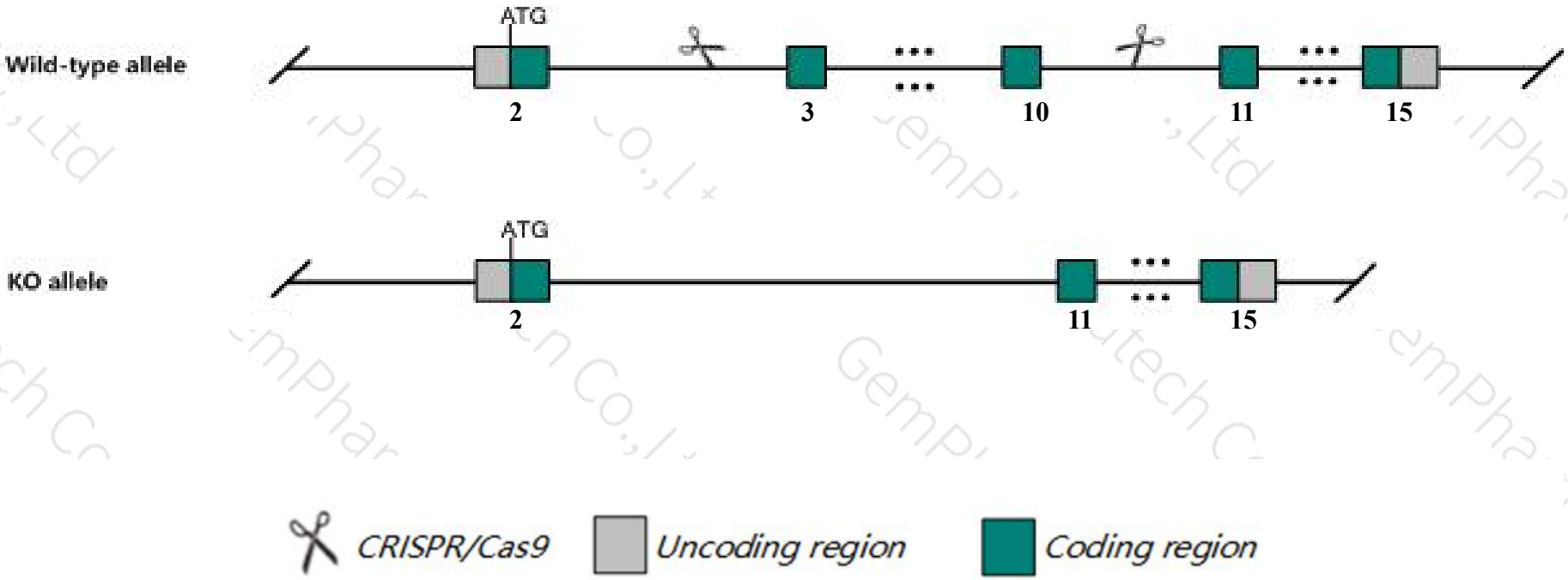
集萃药康 GemPharmatech
GemPharmatech Co.,Ltd

Project Overview

Project Name	***Sema4a***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sema4a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Sema4a* gene has 19 transcripts. According to the structure of *Sema4a* gene, exon3-exon10 of *Sema4a-201* (ENSMUST00000029700.11) transcript is recommended as the knockout region.The region contains 995bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Sema4a* gene. The brief process is as follows: CRISPR/Cas9 syste

Notice

- According to the existing MGI data,Homozygotes for a knock-out allele show no obvious brain defects but exhibit impaired T cell priming and defective Th1 responses. Homozygotes for a gene trap allele show severe retinal degeneration with reduced retinal vessels, depigmentation and dysfunction of both rod and cone photoreceptors.
- The effect on transcript *Sema4a*-203&204&205&208&210 is unknown.
- Transcript *Sema4a*-209&212 may not be affected.
- The knockout region is near to the N-terminal of *Mir7011* gene,this strategy may influence the regulatory function of the N-terminal of *Mir7011* gene.
- The *Sema4a* gene is located on the Chr3. 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）

集萃药康 GemPharmatech

Sema4a sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A [*Mus musculus* (house mouse)]

Gene ID: 20351, updated on 5-Nov-2019

Summary

Official Symbol	Sema4a provided by MGI
Official Full Name	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A provided by MGI
Primary source	MGI:MGI:107560
See related	Ensembl:ENSMUSG00000028064
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	SemB; Semab; AI132332
Expression	Broad expression in colon adult (RPKM 43.5), duodenum adult (RPKM 41.8) and 25 other tissues See more
Orthologs	human all

Genomic context

Location: 3; 3 F1

See Sema4a in Genome Data Viewer

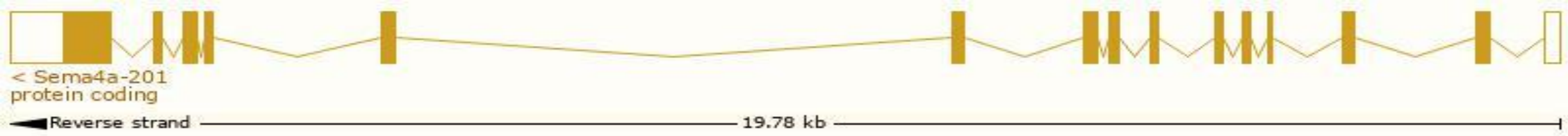
Exon count: 21

Transcript information（Ensembl）

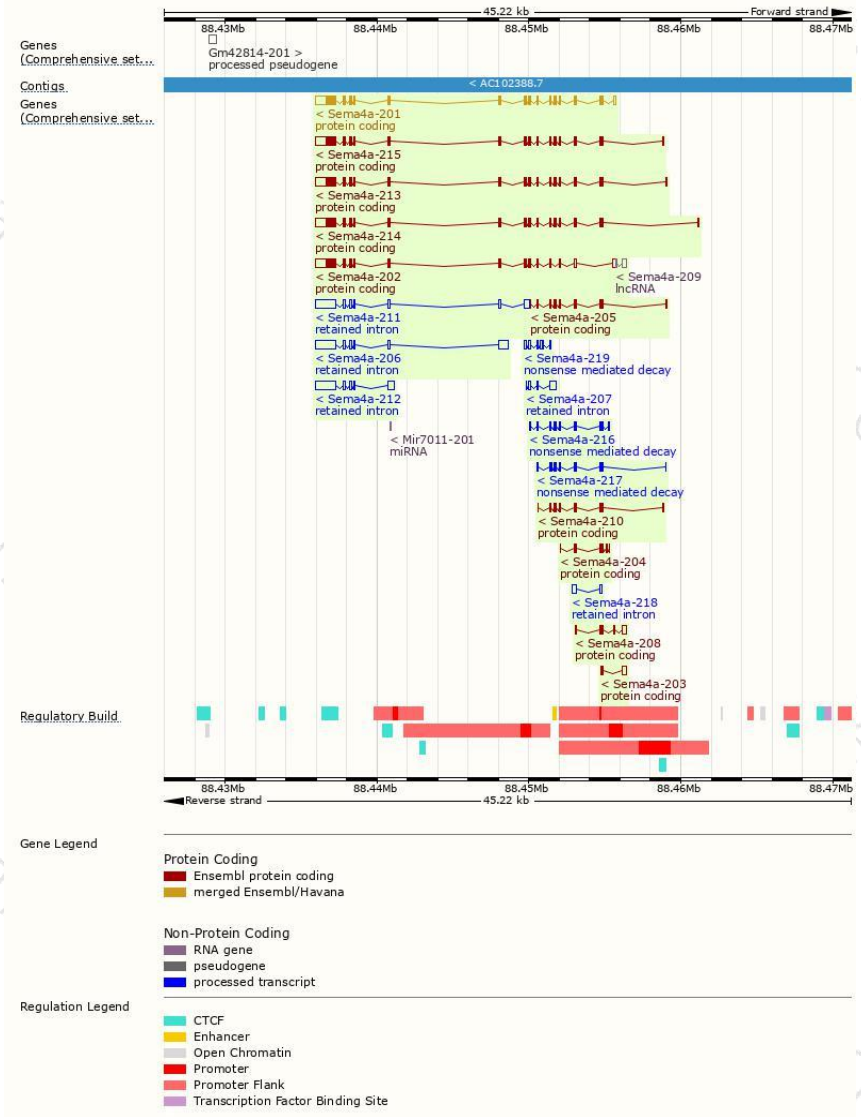
The gene has 19 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sema4a-201	ENSMUST00000029700.11	3205	760aa	Protein coding	CCDS17475	Q62178	TSL:1 GENCODE basic APPRIS P1
Sema4a-213	ENSMUST00000165898.7	3125	760aa	Protein coding	CCDS17475	Q62178	TSL:5 GENCODE basic APPRIS P1
Sema4a-215	ENSMUST00000169222.7	3084	760aa	Protein coding	CCDS17475	Q62178	TSL:5 GENCODE basic APPRIS P1
Sema4a-214	ENSMUST00000166237.7	3046	760aa	Protein coding	CCDS17475	Q62178	TSL:1 GENCODE basic APPRIS P1
Sema4a-202	ENSMUST00000107531.7	2897	628aa	Protein coding	-	D3YWV5	TSL:5 GENCODE basic
Sema4a-205	ENSMUST00000127436.7	845	233aa	Protein coding	-	D3YZ30	CDS 3' incomplete TSL:3
Sema4a-210	ENSMUST00000147200.7	706	203aa	Protein coding	-	D3YUM4	CDS 3' incomplete TSL:5
Sema4a-208	ENSMUST00000141471.1	630	60aa	Protein coding	-	D3YVM6	CDS 3' incomplete TSL:5
Sema4a-204	ENSMUST00000125526.7	506	113aa	Protein coding	-	D3Z336	CDS 3' incomplete TSL:3
Sema4a-203	ENSMUST00000123753.7	385	17aa	Protein coding	-	D3YWK5	CDS 3' incomplete TSL:2
Sema4a-216	ENSMUST00000184487.7	866	170aa	Nonsense mediated decay	-	V9GXF5	TSL:5
Sema4a-217	ENSMUST00000184876.7	748	180aa	Nonsense mediated decay	-	V9GXH9	TSL:5
Sema4a-219	ENSMUST00000185137.7	699	47aa	Nonsense mediated decay	-	V9GWW2	CDS 5' incomplete TSL:3
Sema4a-206	ENSMUST00000135539.7	2487	No protein	Retained intron	-	-	TSL:2
Sema4a-211	ENSMUST00000149145.1	2357	No protein	Retained intron	-	-	TSL:5
Sema4a-212	ENSMUST00000156108.7	2108	No protein	Retained intron	-	-	TSL:2
Sema4a-207	ENSMUST00000135732.7	713	No protein	Retained intron	-	-	TSL:3
Sema4a-218	ENSMUST00000184972.1	465	No protein	Retained intron	-	-	TSL:2
Sema4a-209	ENSMUST00000146921.1	371	No protein	lncRNA	-	-	TSL:3

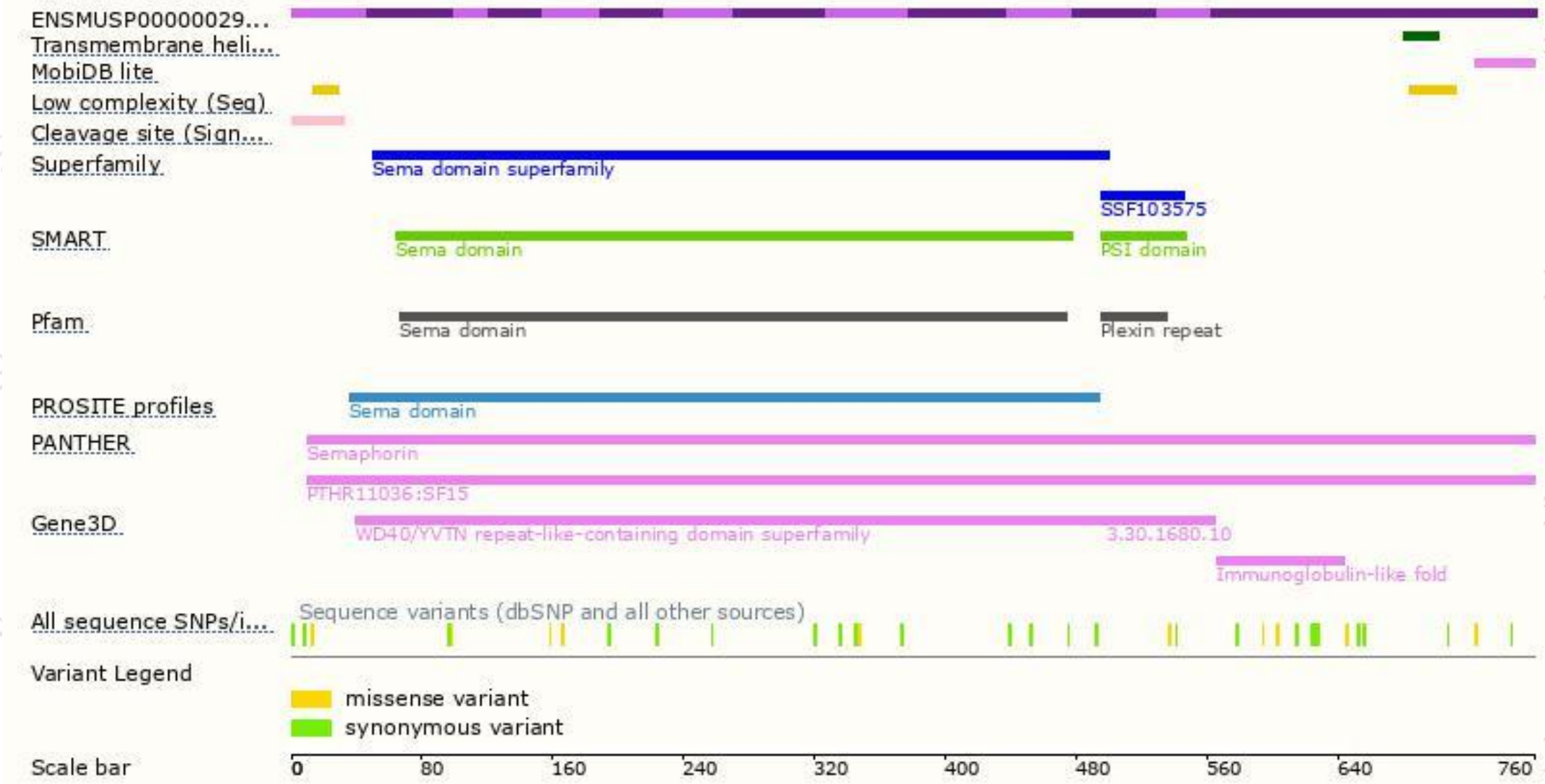
The strategy is based on the design of *Sema4a-201* transcript,The transcription is shown below



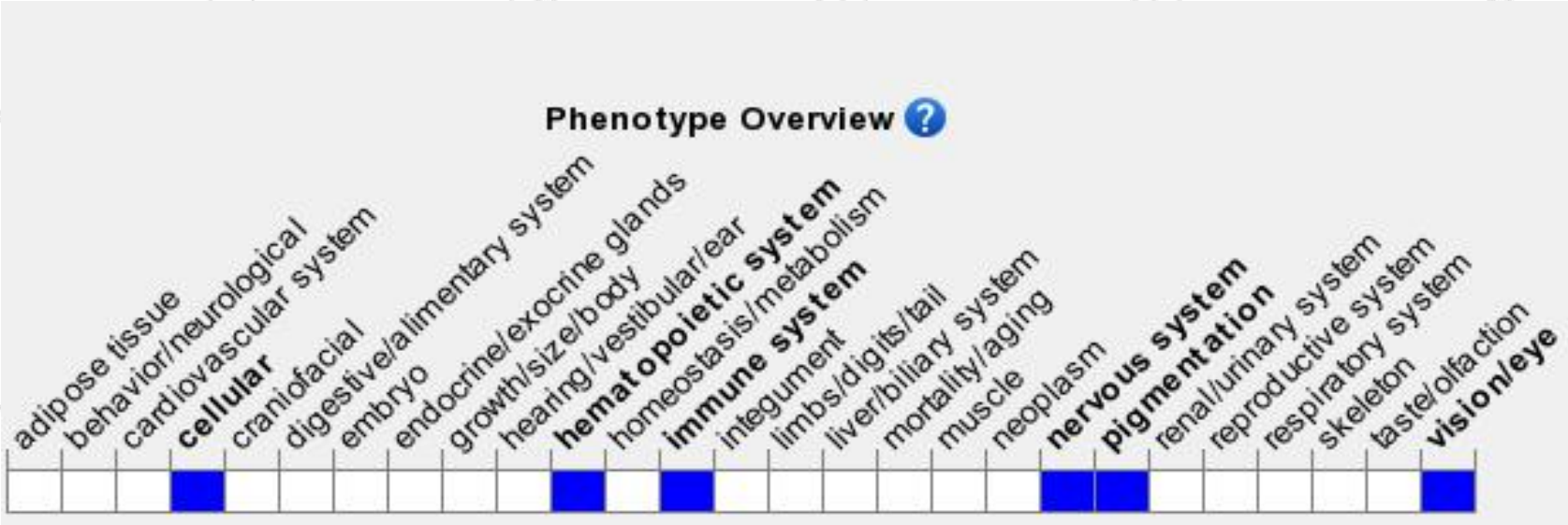
Genomic location distribution



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,Homozygotes for a knock-out allele show no obvious brain defects but exhibit impaired T cell priming and defective Th1 responses. Homozygotes for a gene trap allele show severe retinal degeneration with reduced retinal vessels, depigmentation and dysfunction of both rod and cone photoreceptors.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd

GemPharmatech Co.,Ltd