

Nsmaf Cas9-CKO Strategy

Designer:

Ruirui Zhang

Reviewer:

Huimin Su

Design Date:

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Project Overview

Project Name

Nsmaf

Project type

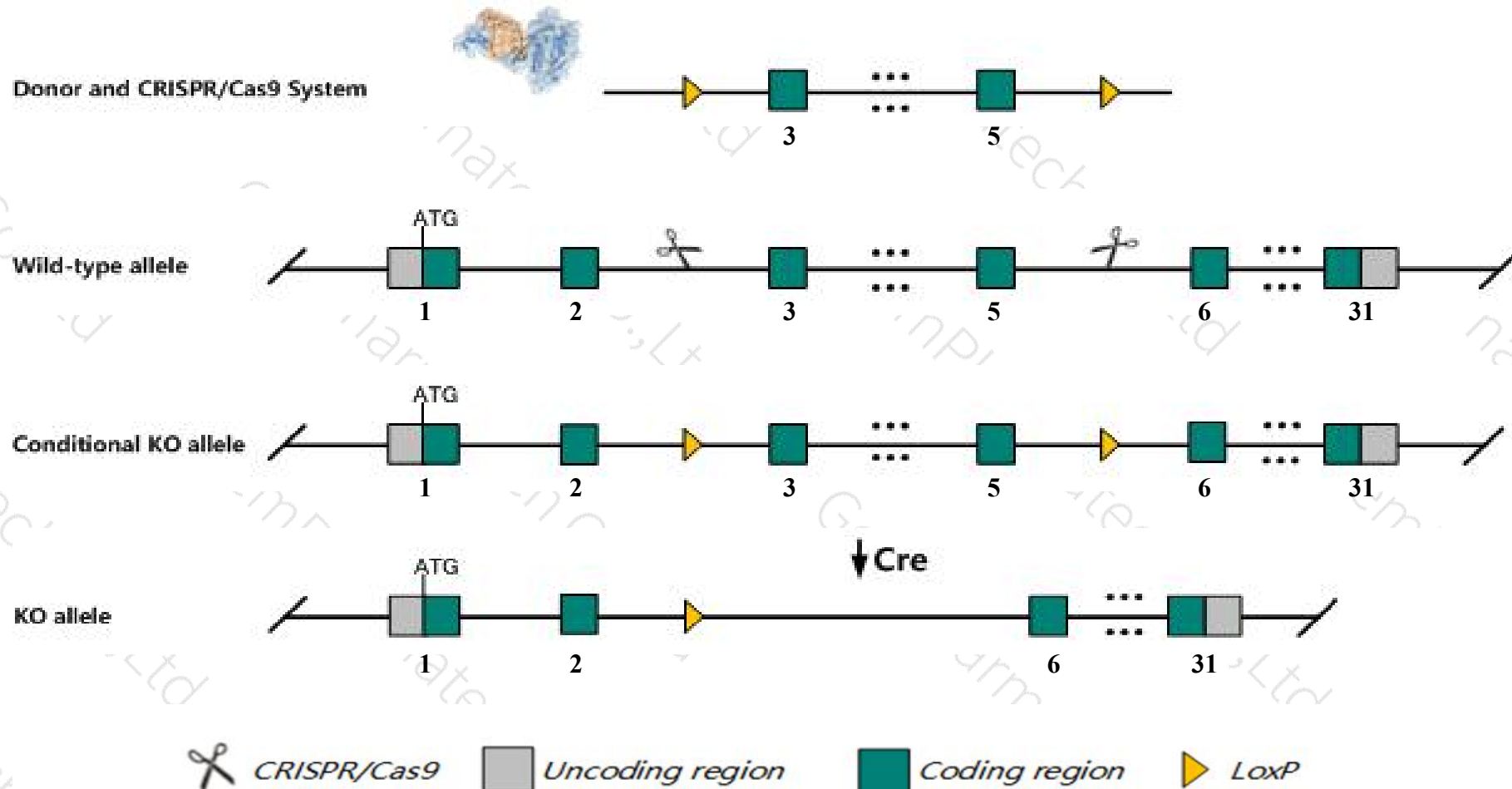
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Nsmaf* gene has 11 transcripts. According to the structure of *Nsmaf* gene, exon3-exon5 of *Nsmaf*-201 (ENSMUST00000029910.11) transcript is recommended as the knockout region. The region contains 184bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Nsmaf* 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.

- According to the existing MGI data, mice homozygous for a targeted null mutation show no gross phenotypic abnormalities but display delayed cutaneous barrier repair. In addition, D-galactosamine-sensitized homozygotes are partially resistant to LPS- and TNF-induced lethality.
- The *Nsmf* 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)

Nsmaf neutral sphingomyelinase (N-SMase) activation associated factor [*Mus musculus* (house mouse)]

Gene ID: 18201, updated on 12-Aug-2019

Summary

Official Symbol Nsmaf provided by [MGI](#)

Official Full Name neutral sphingomyelinase (N-SMase) activation associated factor provided by [MGI](#)

Primary source [MGI:MGI:1341864](#)

See related [Ensembl:ENSMUSG000000028245](#)

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 Fan; AA959567; C630007J05

Expression Ubiquitous expression in bladder adult (RPKM 12.6), ovary adult (RPKM 10.9) and 28 other tissues [See more](#)

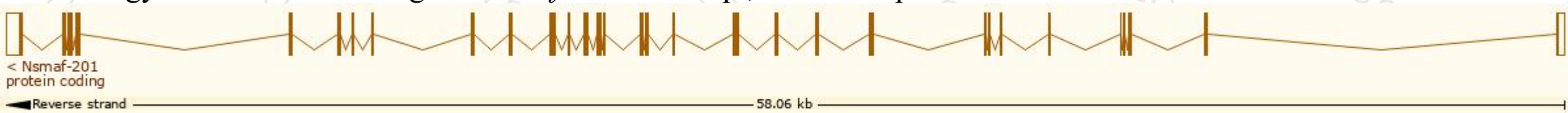
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

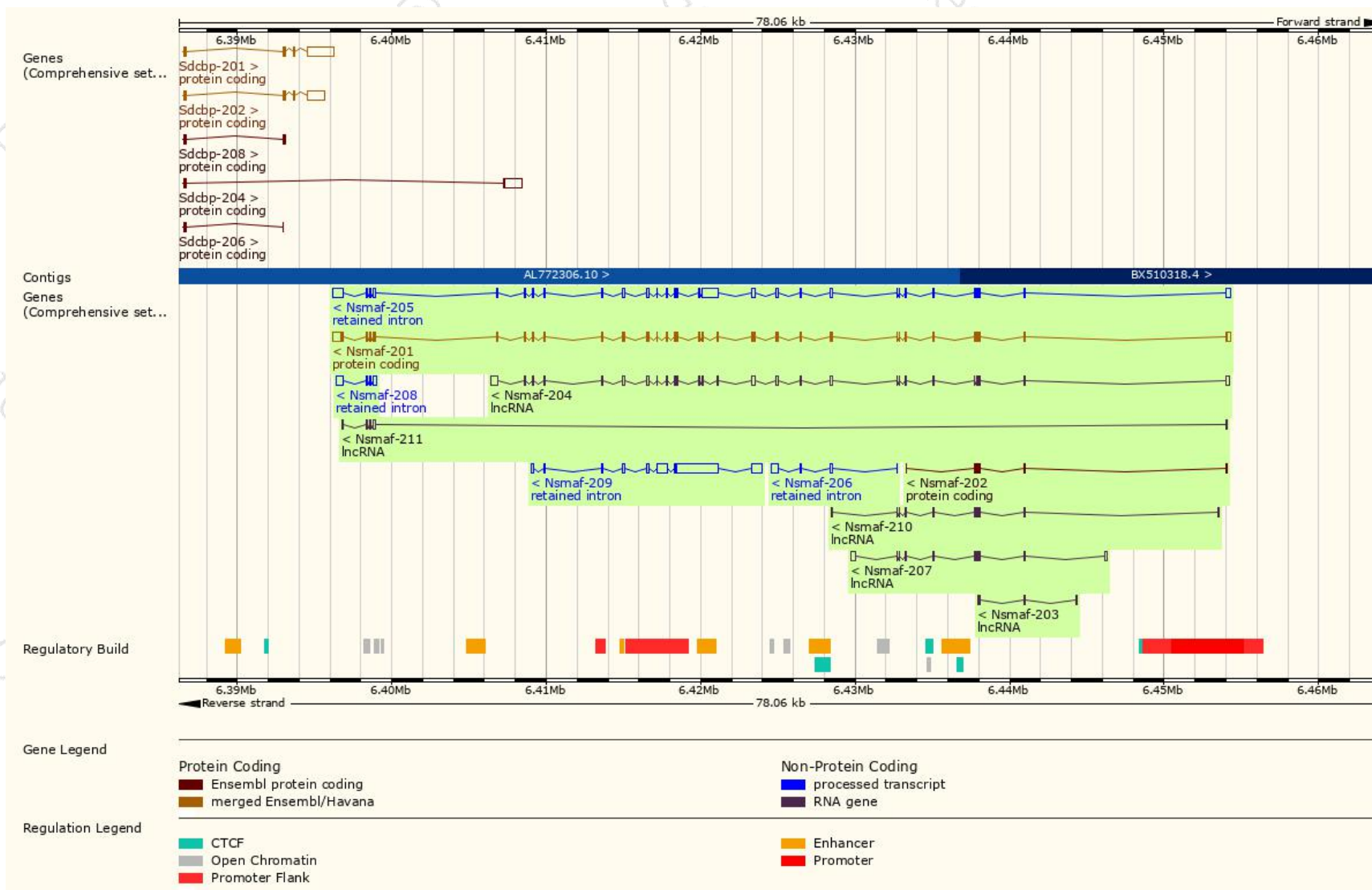
The gene has 11 transcripts,all transcripts are shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲
Nsmaf-201	ENSMUST00000029910.11	3507	920aa	Protein coding	CCDS17952	O35242	TSL:1 GENCODE basic APPRIS P1
Nsmaf-202	ENSMUST00000124344.1	450	132aa	Protein coding	-	A2AKK2	CDS 3' incomplete TSL:5
Nsmaf-203	ENSMUST00000124656.1	294	No protein	lncRNA	-	-	TSL:3
Nsmaf-204	ENSMUST00000143566.7	2870	No protein	lncRNA	-	-	TSL:5
Nsmaf-205	ENSMUST00000143704.7	4423	No protein	Retained intron	-	-	TSL:2
Nsmaf-206	ENSMUST00000143983.1	639	No protein	Retained intron	-	-	TSL:5
Nsmaf-207	ENSMUST00000145399.7	930	No protein	lncRNA	-	-	TSL:1
Nsmaf-208	ENSMUST00000149015.7	818	No protein	Retained intron	-	-	TSL:2
Nsmaf-209	ENSMUST00000156078.1	4705	No protein	Retained intron	-	-	TSL:5
Nsmaf-210	ENSMUST00000156283.7	663	No protein	lncRNA	-	-	TSL:5
Nsmaf-211	ENSMUST00000156715.1	525	No protein	lncRNA	-	-	TSL:3

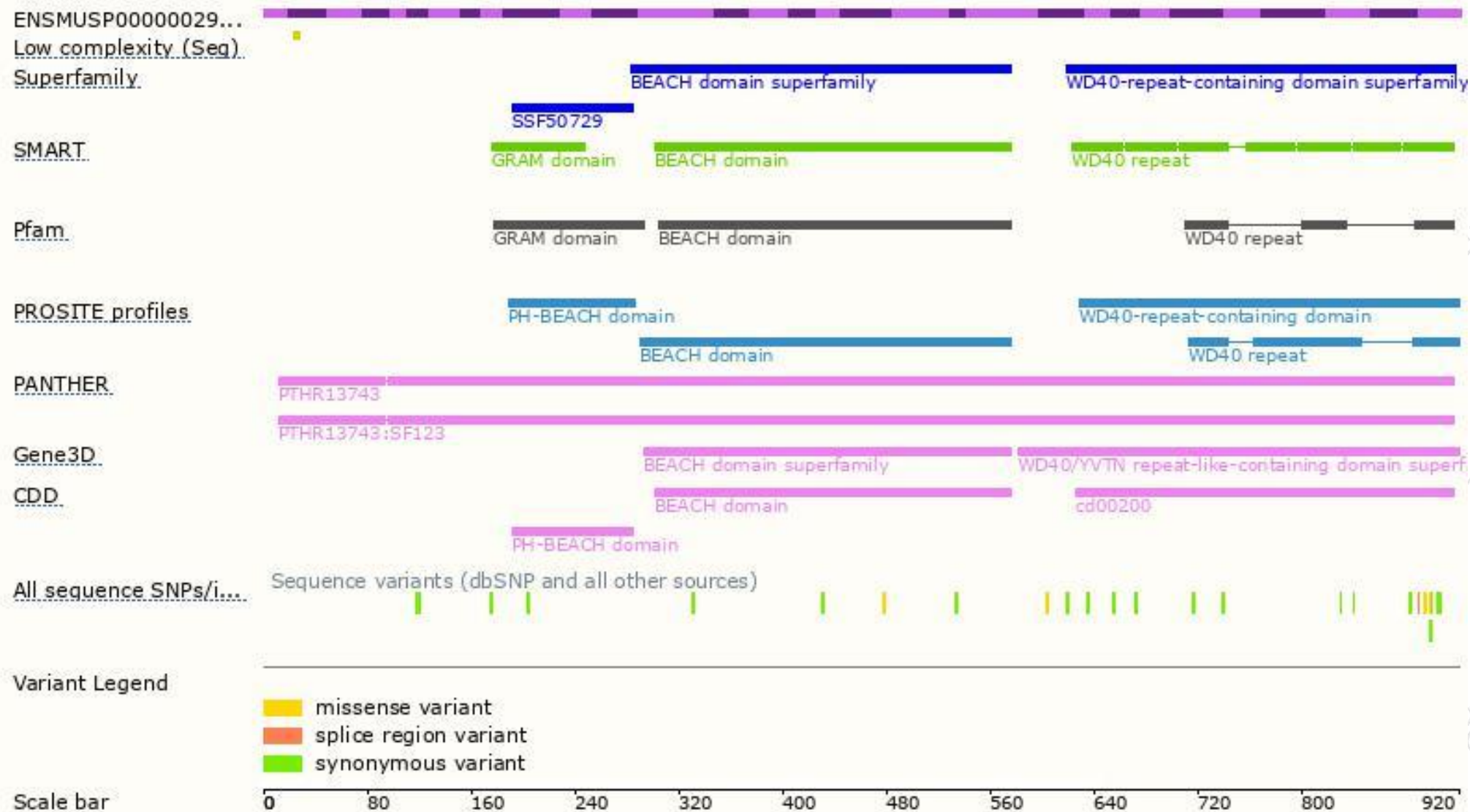
The strategy is based on the design of *Nsmaf-201* transcript,the transcription is shown below:



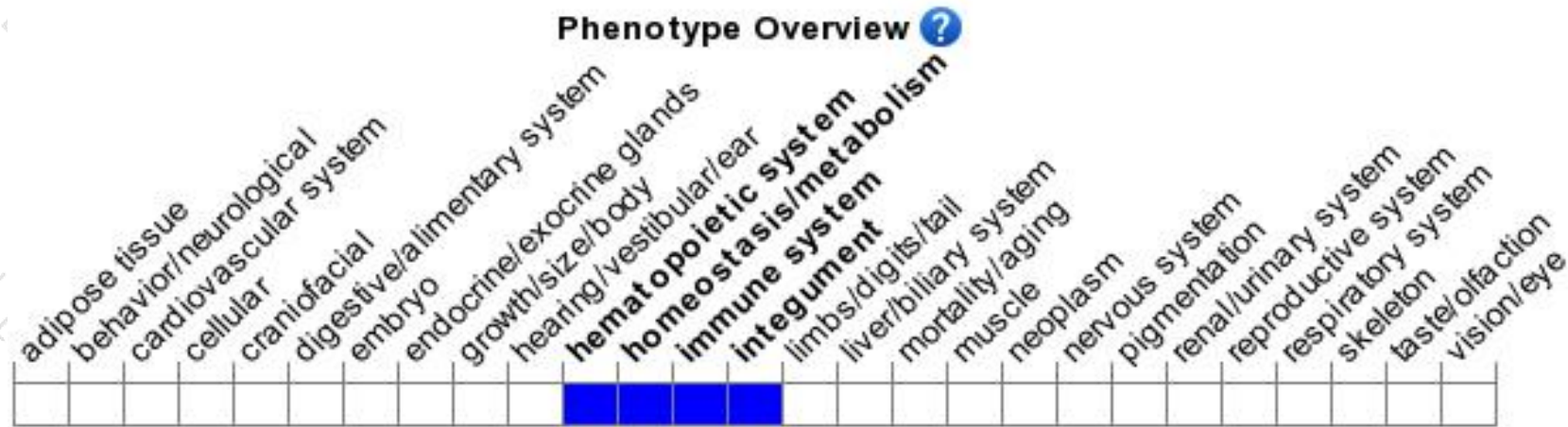
Genomic location distribution



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 targeted null mutation show no gross phenotypic abnormalities but display delayed cutaneous barrier repair. In addition, D-galactosamine-sensitized homozygotes are partially resistant to LPS- and TNF-induced lethality.

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

Tel: 400-9660890

