

Tlr9 Cas9-CKO Strategy

Designer:

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Design Date:

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Project Overview

Project Name

Tlr9

Project type

Cas9-CKO

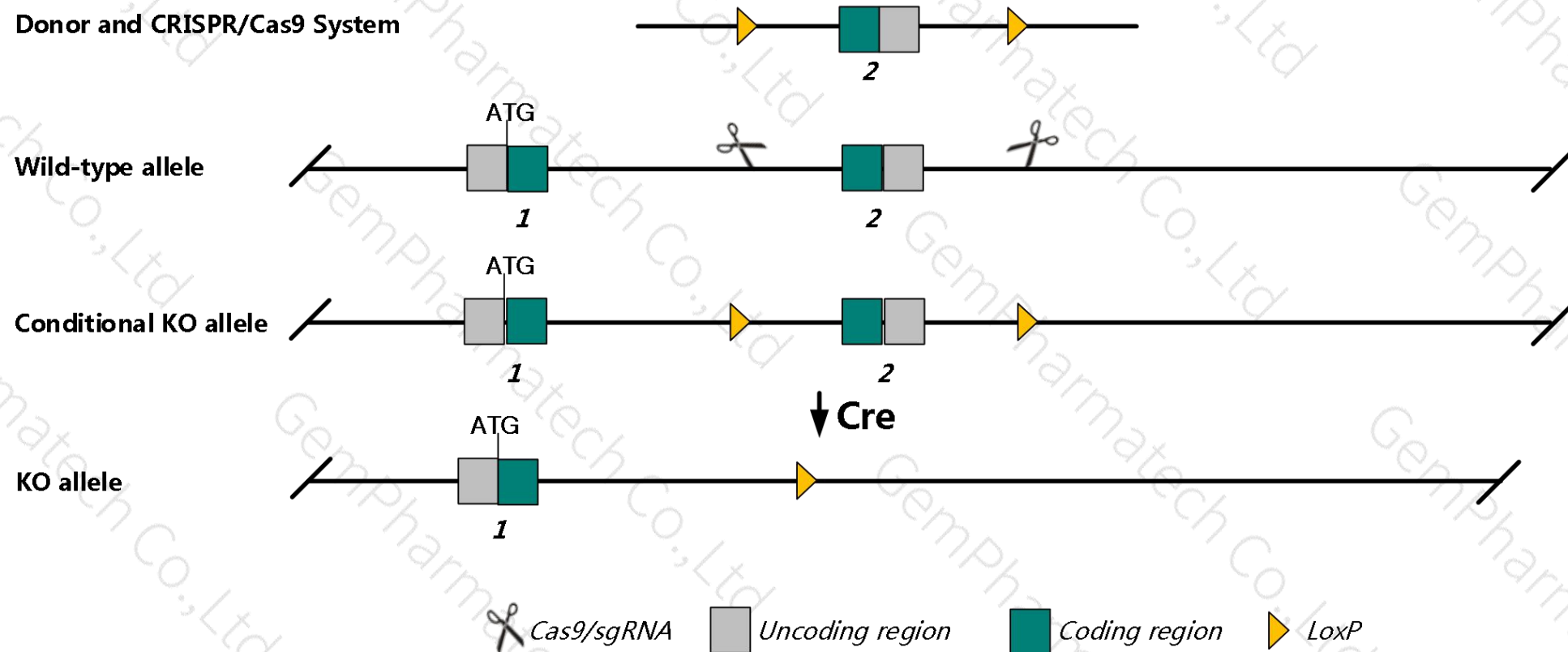
Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tlr9* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System



- The *Tlr9* gene has 1 transcript. According to the structure of *Tlr9* gene, exon2 of *Tlr9*-201 (ENSMUST00000062241.10) transcript is recommended as the knockout region. The region contains the most coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tlr9* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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 or cell types.

- According to the existing MGI data : Nullizygous mice exhibit impaired immune responses to CpG DNA and altered susceptibility to EAE and parasitic infection. ENU-induced mutants may exhibit altered susceptibility to viral infection or induced colitis and impaired immune response to unmethylated CpG oligonucleotides.
- The *Tlr9* gene is located on the Chr9. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Tlr9 toll-like receptor 9 [*Mus musculus* (house mouse)]

Gene ID: 81897, updated on 17-Oct-2018

Summary

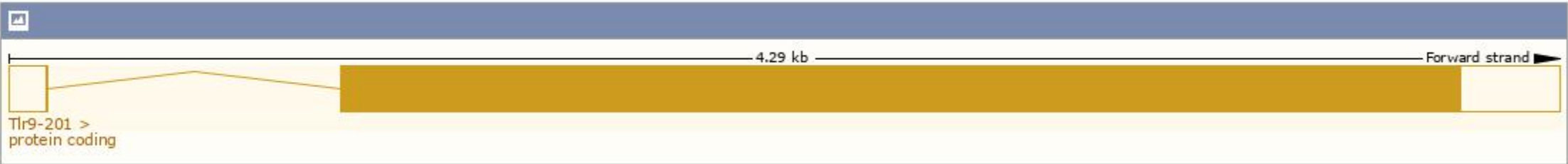
Official Symbol	Tlr9 provided by MGI
Official Full Name	toll-like receptor 9 provided by MGI
Primary source	MGI:MGI:1932389
See related	Ensembl:ENSMUSG000000045322 Vega:OTTMUSG000000049422
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in spleen adult (RPKM 37.8), mammary gland adult (RPKM 11.5) and 5 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

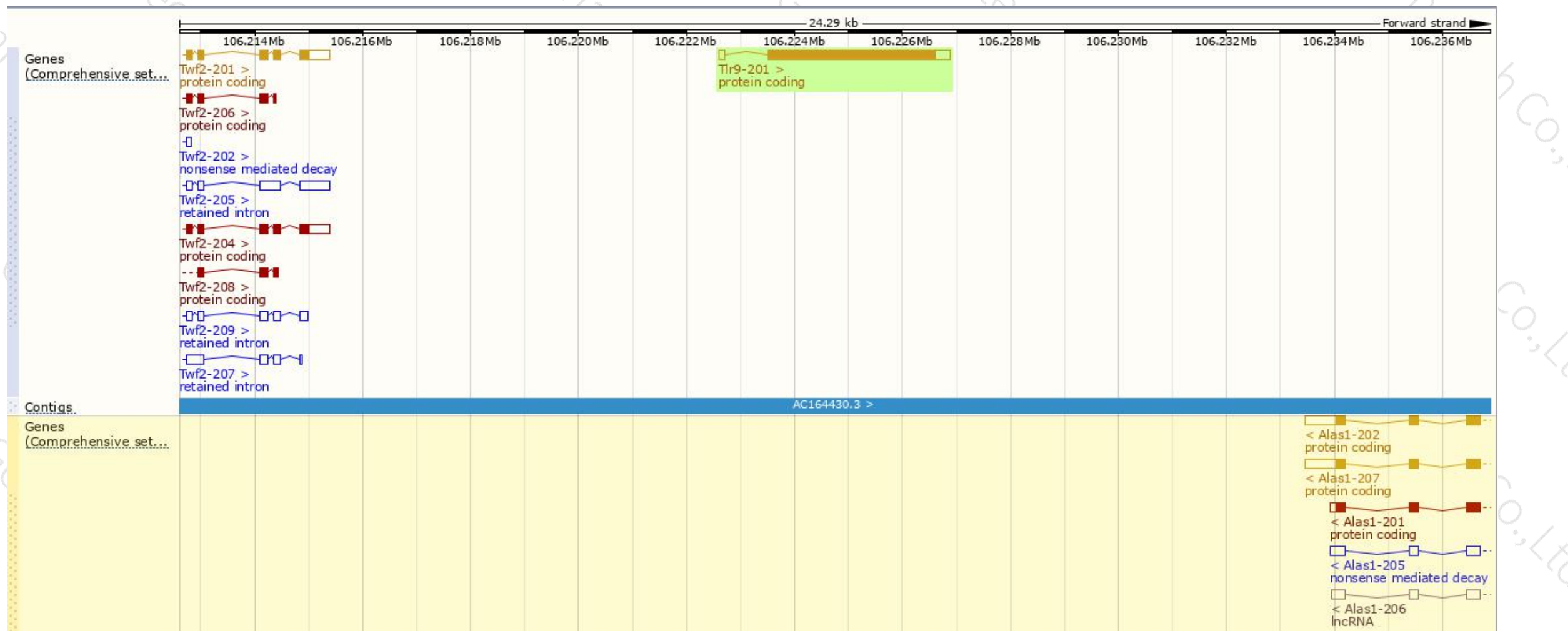
The gene has 1 transcript, and all transcripts are shown below:

Show/hide columns (1 hidden)								Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags	
Tlr9-201	ENSMUST00000062241.10	3478	1032aa	Protein coding	CCDS40755	Q9EQU3	NM_031178 NP_112455	TSL:1	GENCODE basic APPRIS P1

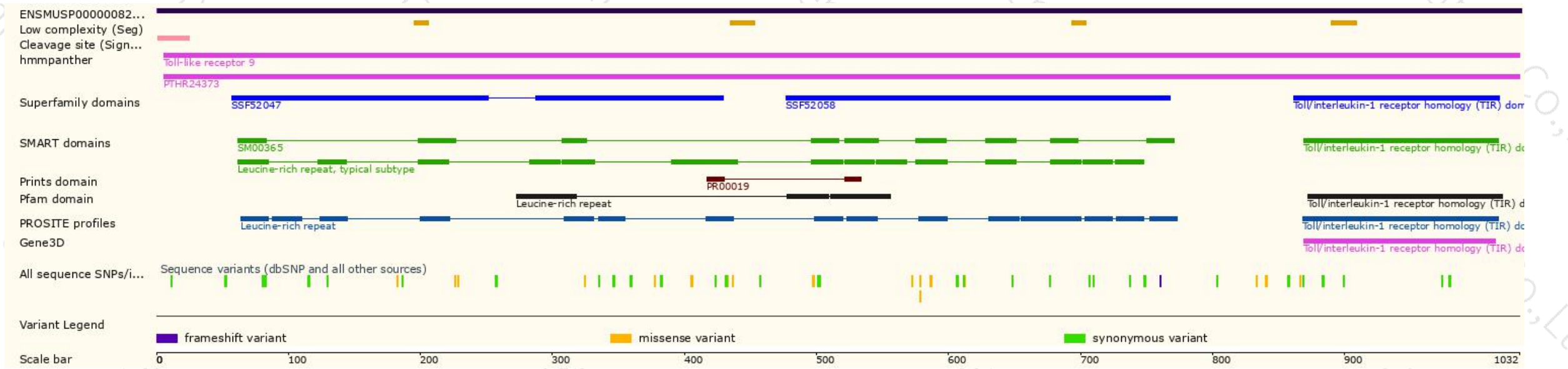
The strategy is based on the design of *Tlr9*-201 transcript, The transcription is shown below



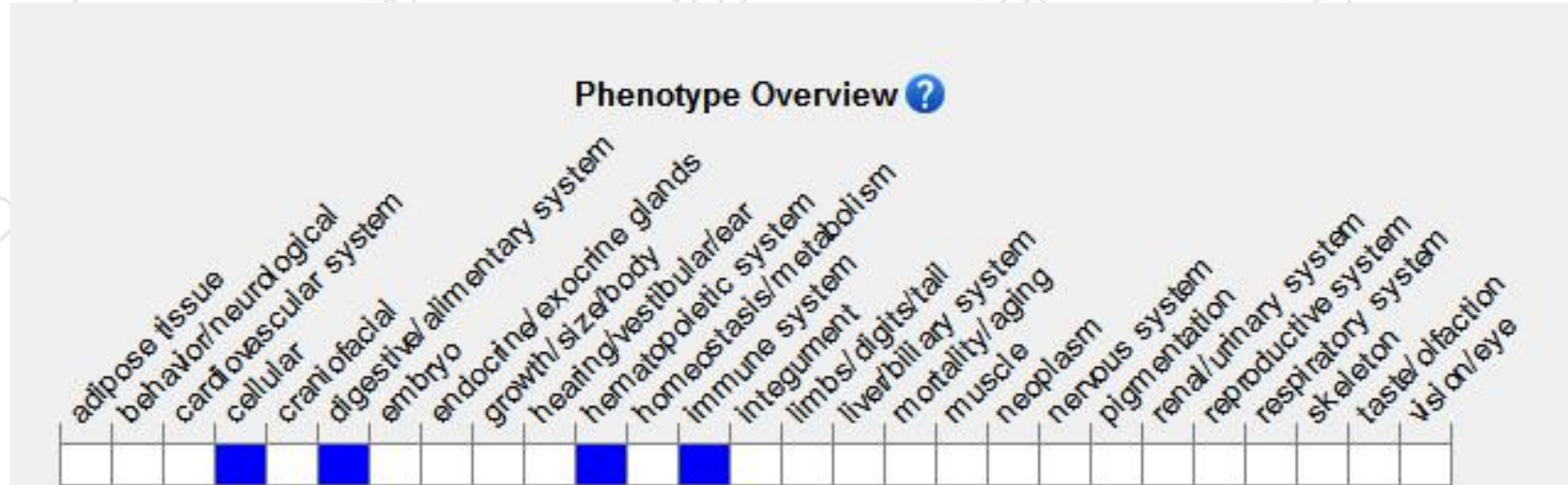
Genomic location distribution



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/>) .

Nullizygous mice exhibit impaired immune responses to CpG DNA and altered susceptibility to EAE and parasitic infection. ENU-induced mutants may exhibit altered susceptibility to viral infection or induced colitis and impaired immune response to unmethylated CpG oligonucleotides.

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