H2-Aa Cas9-CKO Strategy

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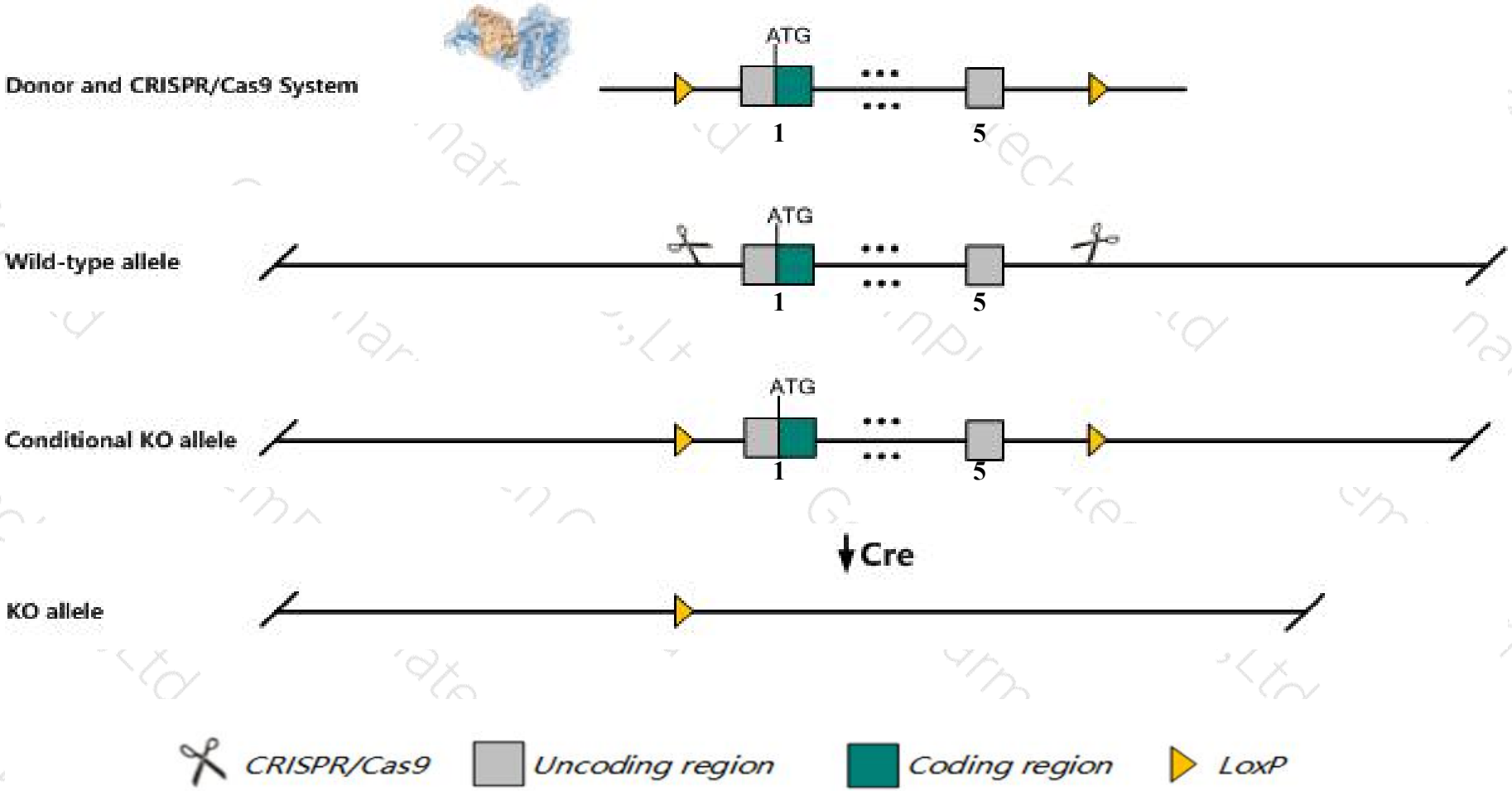
Project Overview

Project Name	***H2-Aa***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *H2-Aa* gene. The schematic diagram is as follows:



Technical routes

- The *H2-Aa* gene has 4 transcripts. According to the structure of *H2-Aa* gene, exon1-exon5 of *H2-Aa-201* (ENSMUST00000040655.13) transcript is recommended as the knockout region.The region contains all the coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *H2-Aa* 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 and cell types.

Notice

- According to the existing MGI data,Mice homozygous for a knock-out allele lack cell surface expression of MHC class II molecules on macrophages and show decreased CD4-positive T cell number, increased CD8-positive T cell number, thymus hyperplasia, enlarged lymph nodes, and altered splenocyte response to staphylococcal enterotoxin B.
- The floxed region is near to the C-terminal of *Gm20513* gene,this strategy may influence the regulatory function of the C-terminal of *Gm20513* gene.
- The *H2-Aa* gene is located on the Chr17. 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）

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H2-Aa histocompatibility 2, class II antigen A, alpha [*Mus musculus* (house mouse)]

Gene ID: 14960, updated on 12-Aug-2019

Summary

Official Symbol	H2-Aa provided by MGI
Official Full Name	histocompatibility 2, class II antigen A, alpha provided by MGI
Primary source	MGI:MGI:95895
See related	Ensembl:ENSMUSG00000036594
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	Ia1; H2Aa; Ia-1; H-2Aa; Aalpha; IAalpha; I-Aalpha
Expression	Biased expression in spleen adult (RPKM 791.4), large intestine adult (RPKM 638.1) and 14 other tissues See more
Orthologs	human all

Genomic context

Location: 17 B1; 17 17.98 cM

See H2-Aa in Genome Data Viewer

Exon count: 5

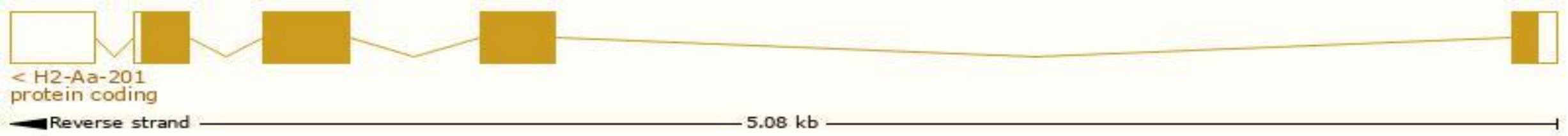
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	17	NC_000083.6 (34282744..34287823, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	17	NC_000083.5 (34419696..34424716, complement)

Transcript information（Ensembl）

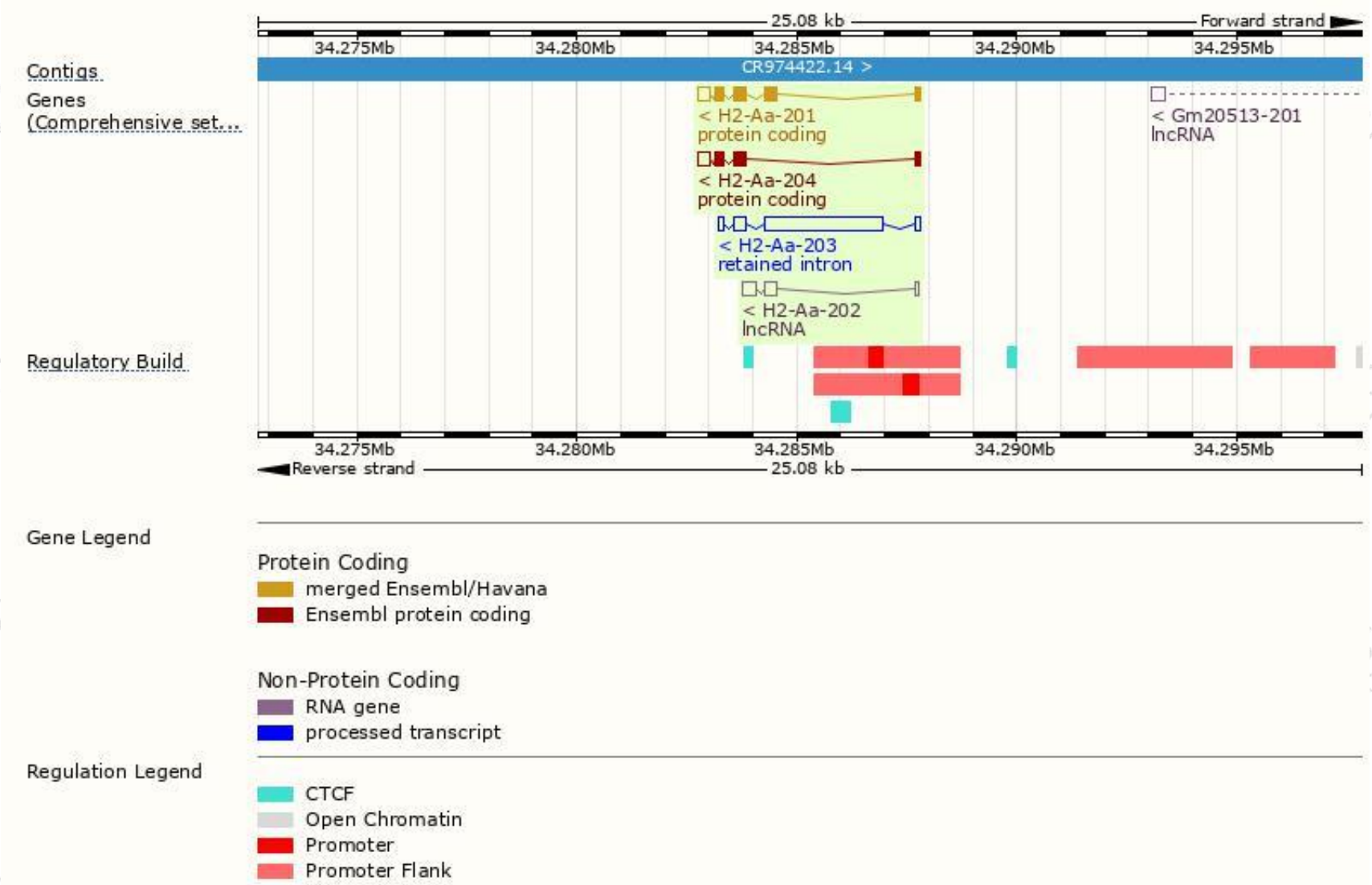
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
H2-Aa-201	ENSMUST00000040655.13	1128	256aa	Protein coding	CCDS28645	P14434	TSL:1 GENCODE basic APPRIS P1
H2-Aa-204	ENSMUST00000174751.1	826	173aa	Protein coding	-	G3UWR9	TSL:3 GENCODE basic
H2-Aa-203	ENSMUST00000173944.2	3223	No protein	Retained intron	-	-	TSL:2
H2-Aa-202	ENSMUST00000165139.2	664	No protein	lncRNA	-	-	TSL:2

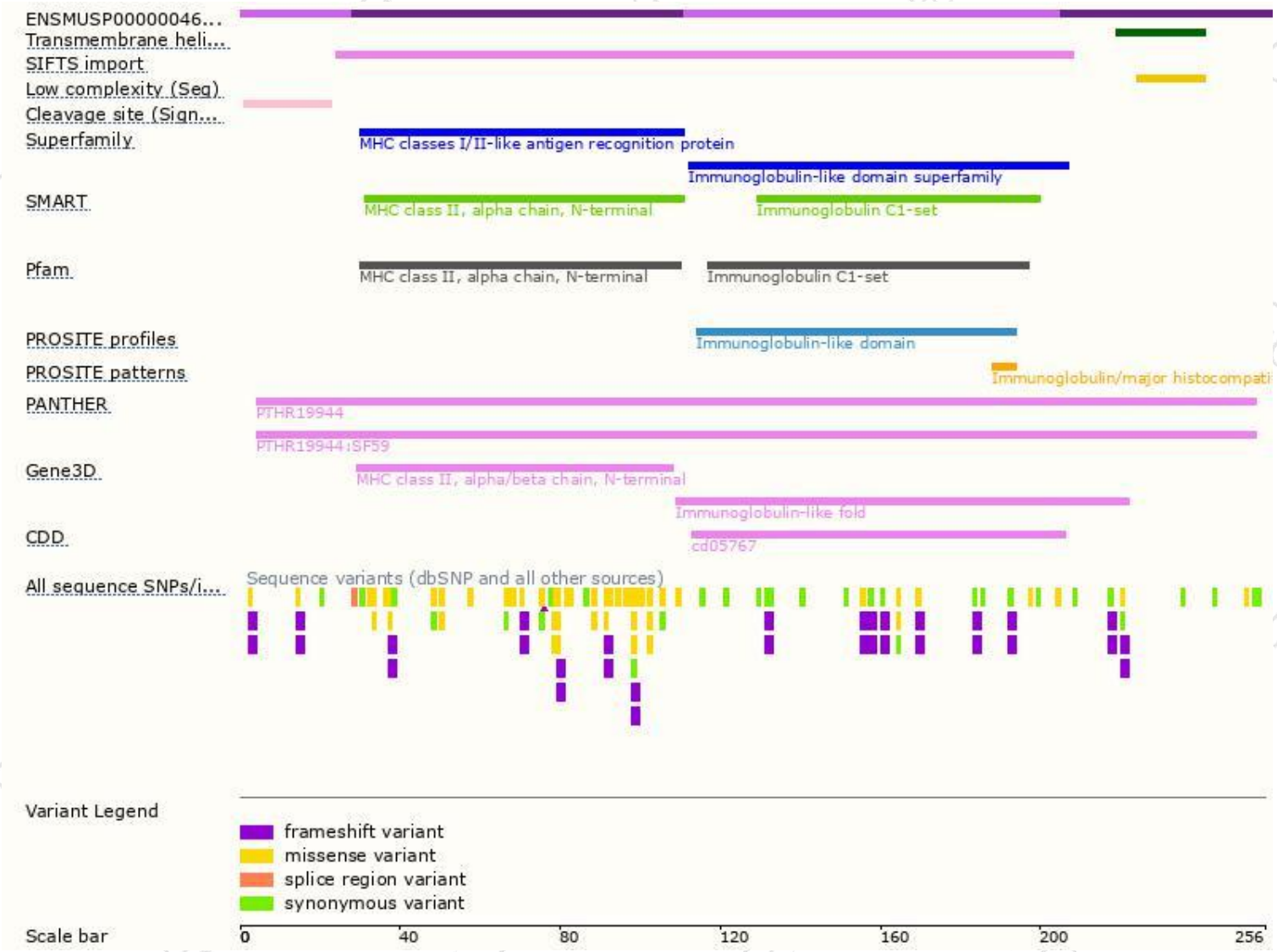
The strategy is based on the design of *H2-Aa-201* transcript,The transcription is shown below



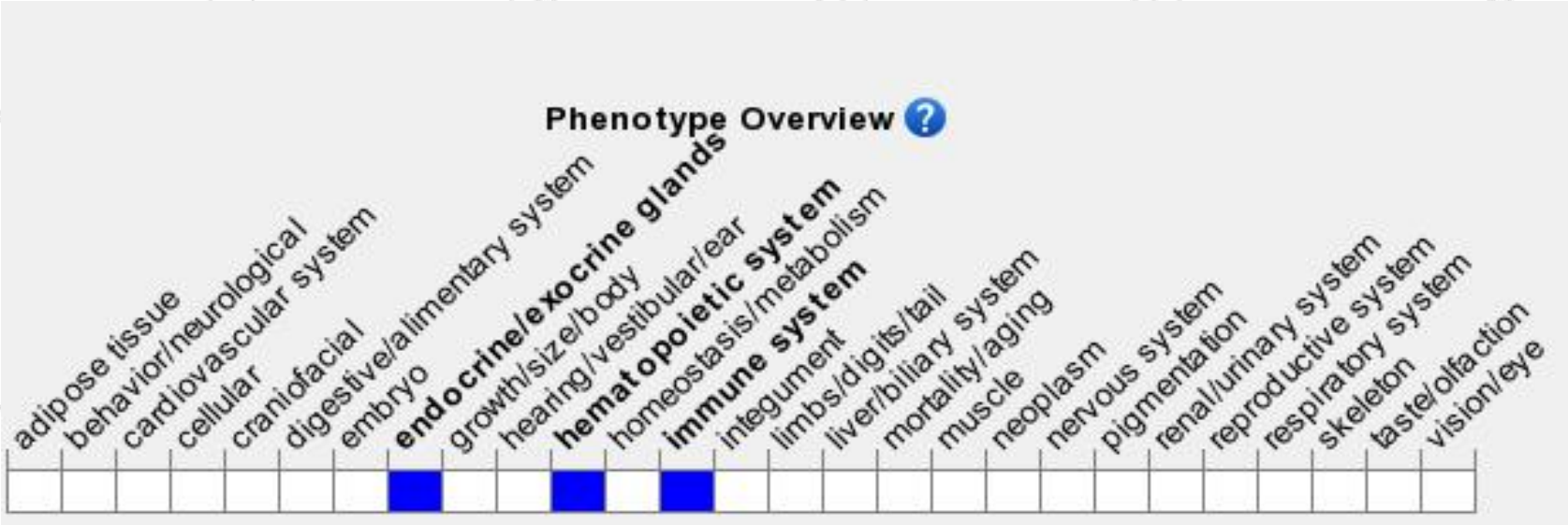
Genomic location distribution



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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